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<p>(54) Title: HUMAN NEURONAL ACID-SENSITIVE CATIONIC CHANNELS, ITS CLONING AND APPLICATIONS</p> <p>(57) Abstract</p> <p>Non-inactivating or slowly inactivating proton-gated cation channels are thought to play an important role in the perception of pain that accompanies tissue acidosis. We have identified a human proton-gated cation channel subunit that has biphasic desensitisation kinetics with both a rapidly inactivating Na^+-selective and a sustained component. The protein shares 84 % sequence identity with the proton-gated cation channel rASIC3 (rDRASIC) from rat sensory neurones. The biphasic desensitisation kinetics and the sequence homology suggest that this clone (hASIC3) is the human orthologue of rASIC3 (rDRASIC). While rASIC3 (rDRASIC) requires very acidic pH (< pH 4.5) for activation of the sustained current, the non-inactivating hASIC3 current starts to be activated when the pH decreases to below pH 6. hASIC3 is an acid sensor and might play an important role in the detection of lasting pH changes in human. We localized the hASIC3 gene to the human chromosome 7q35, 6.4 cRad telomeric from the microsatellite AFMA082XC9.</p>			

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HUMAN NEURONAL ACID-SENSITIVE CATIONIC CHANNELS, ITS CLONING AND APPLICATIONS

BACKGROUND OF THE INVENTION5 *Cross-Reference To Related Patent Applications*

This patent application benefits of and claims the benefit of provisional patent application serial no. 60/095,408, *Identification, Functional Expression and Chromosomal Localisation Of Sustained Human-Proton-Gated Cation Channel*, filed on August 5, 1998, and application serial no. 09/129,758, 10 *Mammal Neuronal Acid Sensing Cationic Channel, Cloning and Application Thereof* filed on August 5, 1998. The said U.S. applications being incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to new families of mammalian, notably 15 human and rat, acidity-sensitive ionic channels. More particularly, the invention relates to the identification and molecular characterization in humans and rats of a new family of proton-activated cationic channels, collectively referred to below as ASIC polypeptides, for Acid Sensing Ionic Channel.
20 The ASIC channels constitute the first members of a group of cationic channels belonging to the family of amiloride-sensitive degenerine sodium channels [6, 11-14], which are activated temporarily by extracellular acidification.

Sensitivity to acid is associated with both nociception [1] and the transduction of taste [2]. The stimulation of sensory neurons by acids is of great importance because acidity accompanies numerous painful inflammatory and ischemic situations. The pain caused by acids is thought to

5 be mediated by the cationic channels present at the level of the sensory neurons which are activated by protons [3-5]. The biophysical and pharmacological properties of the ASIC channels of the invention are similar to those of the proton-activated cationic channels described in the sensory neurons [3, 15, 16]. However, as will be seen in the description below, to

10 date there has been no report of ligand-activated ionic channels simpler than the ASIC channels.

Summary of the Invention

The invention also relates to hybrid cationic channels constituted by the combination of a first protein comprising a proton-activated ionic channel

15 according to the invention with a second proton-activated ionic channel.

The present invention has as its object a nucleic acid molecule coding for a protein constituting a neuronal neuronal cationic channels that is sensitive to amiloride and activated by protons.

The invention also relates to a vector comprising at least one of the

20 preceding nucleic acid molecules, advantageously combined with suitable control sequences, as well as a procedure for production or expression in a cell host of a protein constituting an ionic channel according to the Invention.

The invention also relates to the transformed cells expressing ASIC cation channels and/or their derivatives obtained according to the preceding methods.

The present invention also relates to application of the ASIC channel for studying pathological modifications that may lead to neuronal degenerations. The invention this also relates to the pharmaceutical preparations comprising as an active ingredient, at least one of these proteins of the invention.

Other characteristics and advantages of the invention will be seen in the description below related to research activities that led to the demonstration and the characterization of the ASIC channel.

This invention can be further understood with reference to the Figures, discussed next and in the Examples.

15

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents the alignment of the sequences of the rat ASIC proteins (at top) and human ASIC proteins (at bottom) of sequences SEQ ID NO: 1 and SEQ ID NO: 2.

20 **Figure 2** represents a comparison of the protein sequence of the rASIC1A channel with the sequence of other ionic channels:

Figure 3 represents the phylogenetic tree of the proteins of the subunits α NaCh, β NaCh, γ NaCh, δ NaCh of the amiloride-sensitive sodium channel and of the degenerines MEC-4, MEC-10 and DEG-1 of *C. elegans*.

Figure 4 represents the topology proposed for this latter family of ionic channels [30].

Figure 5 shows the biophysical properties of the proton-activated rASIC1A channel.

5 **Figure 6** shows the effect of Ca^{2+} and of amiloride on the rASIC1A current.

Figure 7 shows the tissue distribution of ASIC1A channel mRNA.

Figure 8 shows the *in situ* hybridization.

10 **Figure 9** shows the alignment of the deduced protein sequences of hASIC3 and rASIC3.

Figure 10 shows the pH dependence and pharmacology of hASIC3.

Figure 11 shows the selectivity and single channel properties of hASIC3.

15 **Figure 12** shows the human chromosomal localization of the hASIC3 gene.

IDENTIFICATION OF THE AMINO ACID AND DNA SEQUENCES

SEQ ID NO: 1 represents the sequence of 526 amino acids of the protein of the rASIC1A channel deduced from the cDNA sequence of the rat.

20 **SEQ ID NO: 2** represents the partial sequence of 514 amino acids of the protein of the hASIC1A channel deduced from the partial sequence of human cDNA.

SEQ ID NO: 3 represents the sequence of 512 amino acids of the protein of the hASIC2A channel deduced from the sequence of human cDNA.

SEQ ID NO: 4 represents the sequence of 559 amino acids of the protein of the rASIC1B channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

SEQ ID NO: 5 represents the sequence of 533 amino acids of the 5 protein of the rASIC3 channel and the sequence of DNA coding for that protein.

SEQ ID NO: 6 represents the sequence of 563 amino acids of the protein of the rASIC2B channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

10 SEQ ID NO: 7 represents the sequence of 533 amino acids of the protein of the hASIC3 channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

DETAILED DESCRIPTION OF THE INVENTION

15 The present invention has as its object and rat proteins constituting neuronal cationic channels that are sensitive to amiloride and which are activated by protons. The invention relates to proteins constituting the ASIC family of cation channels, or functionality equivalent derivatives of these proteins.

20 Such derivatives are those polypeptides whose sequence includes a modification and/or a suppression and/or an addition of one or more amino acid residues, as long as this modification, suppression, and/or addition does not alter the functional and structural properties of the ASIC channel, princi-

pally its activation by protons. Indeed, three different ASIC polypeptides, ASIC1, ASIC2 and ASIC3, in both rat and human, are described herein. In addition, the transcripts encoding ASIC1 and ASIC2 are alternatively spliced, which generates additional functional derivatives of the ASIC1 and ASIC2

5 proteins (ASIC1A and 1B, ASIC2A and ASIC2B, respectively). Other functional derivatives of the ASIC proteins and/or other forms of the ASIC polypeptides generated by alternative splicing of the ASIC mRNA transcripts are considered to be within the scope of the present invention. Such proteins and their functional derivatives can be analyzed by an expert in the field

10 using the techniques described in the Examples included herein, which make it possible to demonstrate the biophysical and pharmacological properties of the ASIC channels.

Further examples of functional derivatives of the ASIC channels are as follows: The human and rat ASIC1A proteins (hASIC1A and rASIC1A, SEQ ID 15 Nos. 1 and 2, respectively) are considered to be functionally equivalent. The amino acid sequences of these two proteins are highly homologous, but they are not identical. Thus, substitutions can readily be introduced within the primary sequence of ASIC proteins without influencing their basic functional characteristics.

20 Another example of such a functionally equivalent derivative is the protein constituting a cationic channel previously designated MDEG [14] or BNaCl [20], designated herein as rASIC2A. The amino acid sequence of rASIC2A is represented in the annexed list of sequences under number SEQ

ID NO: 3. rASIC2A has been described as a mammalian cationic channel which is sensitive to amiloride and which is activated in *C. elegans* by mutations that result in neurodegeneration. The rASIC2A channel is a structurally similar to the ASIC1A channel, exhibiting approximately 67% homology 5 in their amino acid sequences. Cation transport by both polypeptides is sensitive to amiloride and regulated by acid. However, the electrophysiological properties of these two channels are different because they are not activated by the same pH changes. Thus, the range of sensitivity of rASIC2A ($EC_{50} = 4.05$) is different from that of ASIC1A ($EC_{50} = 6.2$). Other 10 functionally equivalent proteins that may exhibit different electrophysiological properties are also considered to be within the scope of the invention.

It has been shown that the rASIC2A channel is activated by the same mutations as those causing neuronal degeneration in *C. elegans*. Thus, like the hyperactive mutants of *C. elegans*, the active mutants of rASIC2A are 15 responsible for cell death. This indicates that the acquisition of function by this neuronal ionic channel could be associated with various forms of neuronal degeneration in mammals, notably of rodents and humans. However, no normal physiological function of rASIC2A was known until the demonstration of its activation by protons in accordance with the cationic 20 channels of the present invention.

Other examples of proteins constituting a neuronal cationic channel that are sensitive to amiloride and activated by protons according to the invention are presented below

- A channel designated ASIC1B, whose sequence of 559 amino acids is represented in the annexed list of sequences under number SEQ ID NO: 4. ASIC1B is a splicing variant of the ASIC1A channel cloned from the rat brain by degenerated PCR. The first 185 amino acids are replaced by a new sequence of 218 amino acids which is underlined in SEQ ID NO: 4.

5 - A channel designated rASIC2B. rASIC2B is a splicing

variant of rASIC2A and is represented by SEQ ID No. 6.

- A channel designated rASIC3, whose sequence of 533 amino acids is represented in the list of sequences under number SEQ ID NO: 5. rASIC3 10 was cloned from sensory neurons from the rat using a partial sequence from the data banks (Expressed Sequence Tag with accession number W62694).

The properties of rASIC3 are as follows:

a) It is expressed in the sensory neurons but not in the brain.
b) Its expression in *Xenopus* oocytes or in mammalian cells 15 allows recording of a proton-activated sodium current which presents two components: a component activating and inactivating itself rapidly, and a component activating itself more slowly and not inactivating itself. The two components are selective for Na^+ . A proton-activated cationic channel that does not inactivate itself was implicated in the prolonged sensation of pain 20 caused by acidosis.

- A channel designated hASIC3, which is represented by SEQ ID No. 7. This protein is a novel human proton-gated cation channel subunit that has biphasic desensitisation kinetics, with both a rapidly inactivating Na^+ -

selective and a sustained component. The protein shares 84% sequence identity with the proton-gated cation channel rASIC3 from rat sensory neurons.

The invention also relates to hybrid cationic channels, or channels 5 constituted by the combination of a first protein comprising a proton-activated ionic channel according to the invention with a second protein comprising a proton-activated ionic channel. Advantageously, the said second protein is also a protein comprising a proton-activated ionic channel according to the invention. An example of such a combination is illustrated by the combination 10 of the ASIC1A, ASIC2A or ASIC3 channel with the ASIC2A channel. Such hybrid channels exhibit a third range of pH sensitivity (e.g., with ASIC: EC₅₀ = 4.8). Another example of such a hybrid channel is the combination of the ASIC1A, ASIC1B, ASIC2A or ASIC3 channels with the the ASIC2B channel.

ASIC2B is a channel that was cloned from the rat brain using a partial 15 mouse sequence accessible in the data banks (Expressed Sequence Tag with accession number W50528) and whose sequence of 563 amino acids is represented in the annexed list of sequences under number SEQ ID NO: 6. ASIC2B is a splicing variant of ASIC2A. The first 185 amino acids are replaced by a new sequence of 236 amino acids which is underlined in SEQ ID 20 NO: 6. ASIC2B is expressed in the brain and in the sensory neurons of the dorsal root ganglia.

ASIC2B expressed alone in *Xenopus* oocytes or in mammalian cells does not form a proton-activated cationic channel. However, it can combine

with ASIC2A or ASIC3 to form proton-activated heteromultimeric channels with modified properties. The activation pH of the channel formed after the co-expression of ASIC2A and ASIC2B differs from the channel formed by ASIC2A alone. After expression of ASIC2A and ASIC2B in COS cells, the

5 current has not reached its maximum value at pH 3 whereas the current induced by ASIC2A alone is saturated at a pH between 4.5 and 4.0. In addition, the inactivation kinetics and the ionic selectivity of the channel formed after the co-expression of ASIC2A and ASIC2B are clearly different from those of ASIC2A alone. A current appears which inactivates itself slowly

10 and is barely selective for Na^+ and K^+ .

In another example, the sodium current obtained after expression of ASIC3 becomes non-selective (it does not differentiate between sodium and potassium) when ASIC2B is co-expressed with ASIC3. This new property is similar to that of the proton-activated cationic channel which is implicated in

15 the prolonged sensation of pain caused by acidosis. It is very probable that ASIC3 and ASIC2B are part of this channel.

The amino acid sequence homologies of the proteins constituting the ASIC1A, ASIC1B channels cited according to the invention are presented in Table 1 below.

20

Table 1

Channel	ASIC 1B	ASIC 1A	ASIC2B	ASIC2A	ASIC3
ASIC1B	100	80	56	61	52

ASIC1A		100	59	68	53
ASIC2B			100	78	48
ASIC2A				100	51
ASIC3					100

5

Polyclonal or monoclonal antibodies directed against at least one protein constituting an ion channel of the invention and/or against a hybrid channel as described above can be prepared by the classic methods described in the literature. The antibodies are useful for investigating the presence of the 10 ionic channels of the invention in various human and animal tissues, and may also be used to inhibit or activate an ASIC channel and/or its derivatives *in vivo*. Such an application may be useful for the treatment of diseases arising from defective ASIC cation transport.

The present invention also has as its object a nucleic acid molecule 15 coding for a protein constituting a neuronal cationic channel that is sensitive to amiloride and activated by protons. More particularly, the invention relates to a nucleic acid molecule comprising at least one sequence coding for a protein constituting the ASIC1A, ASIC1B, ASIC2A, ASIC2B, or ASIC3 cation channels from human or rat.

20 The invention also relates to a vector comprising at least one of the preceding nucleic acid molecules, advantageously combined with suitable control sequences, as well as a procedure for production or expression in a cell host of a protein constituting an ionic channel according to the invention. The preparation of these vectors as well as the production or expression of the

channels of the invention in a competent host cell can be accomplished by established methods known to experts in the field.

For example, the expression and production of a protein constituting a cationic channel according to the invention can be accomplished by:

5 - transferring a nucleic acid molecule of the invention or a vector containing said molecule into a competent host cell,

- culturing said host cell host under conditions allowing expression of the ionic channels of the invention.

- isolating the proteins constituting the ionic channels of the invention.

10 The host cell employed in the preceding methods can be selected from among the prokaryotes or the eukaryotes and notably from among the bacteria, yeasts or cells of mammals, plants or insects.

The vector used is selected in relation to the host to which it will be transferred; any vector such as a plasmid can be used.

15 The invention also relates to the transformed cells expressing ASIC cation channels and/or their derivatives obtained according to the preceding methods. These cells are useful for screening to identify substances that are capable of modulating cation transport by these polypeptides and hence, the perception of acidity with regard to both nociception and taste transduction. This 20 screening is implemented by bringing variable quantities of a substance to be tested into contact with cells expressing the ASIC channels and determining the effects of said substance on the currents of said cation channels. These screenings allow for the identification of new drugs that are useful in the

treatment or prevention of pain. They also enable the identification and investigation of agents that modulate acid taste. In addition, these methods are useful for identifying substances that block, or can inhibit neurodegeneration induced by hyperexpression of these channels. The substances which are 5 isolated and detected by means of the methods above are also part of the invention. The ASIC channels clearly have ionic selectivity properties, notably with regard to their selective permeability by sodium, potassium and calcium, which endows them with excitotoxic properties when hyperstimulated.

A protein constituting an ASIC neuronal ionic channel can also be 10 useful for developing drugs intended for the treatment or prevention of pathologies entailing the painful perception of acidity which intervenes in inflammatory diseases, ischemias and a certain number of tumors. The invention thus also relates to pharmaceutical compositions comprising as active ingredients, at least one protein constituting an ionic channel according to the 15 invention.

A nucleic acid molecule coding for a protein constituting an ASIC channel or a derivative thereof, or a vector comprising this nucleic acid molecule or a cell expressing ASIC channels are also useful for the preparation of transgenic animals. These can be animals superexpressing said channels, but also "knock- 20 out" animals, i.e., animals deficient in the expression of these channels or of the cation transport activity of the ASIC channels. These transgenic animals are prepared by methods known to the expert in the field, and enable the

development of live models for studying animal pathologies associated with ASIC channels.

The nucleic acid molecules of the invention or the cells transformed by said molecule can thus be used for genetic therapy to compensate for a 5 deficiency in the ASIC channels at the level of one or more tissues of a patient.

The invention thus relates also to a drug comprising nucleic acid molecules of the invention or cells transformed by said nucleic acid molecules for the treatment of pathology involving the ASIC channels or their derivatives.

In addition to the property of being activated by protons and the resultant 10 applications described above relating to the perception of acidity, the ASIC channels, and particularly ASIC channels that have genetic mutations, may be involved in some neurodegenerative processes. The death of certain neurons is characteristic of many types of neuronal degenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic 15 lateral sclerosis and cerebellar ataxia. Studies of such neurodegenerative processes have identified only a few deficient genes that may be responsible for or associated with these diseases. It is likely that many more important genes remain to be identified. The primitive neural network of the nematode *C. elegans* constitutes a good model of neuronal development and death. The 20 hereditary degeneration in *C. elegans* can be due to mutations of the genes deg-1, *mec-4* and *mec-10*. These genes exhibit homology with the subunits of amiloride-sensitive sodium channels. In addition, the functional expression of the *mec-4* chimeras of the epithelial sodium channel, suggest that these genes

are ionic channels whose acquisition of function is the cause of neuronal degeneration.

The present invention thus also relates to application of the ASIC channel for studying these pathological modifications that may lead to neuronal 5 degenerations. The techniques employed for these applications, for example for drug screening, are similar to those described above for the investigation of taste-modulating agents and analgesic agents.

In addition, a protein constituting an ASIC neuronal ionic channel, an agonist or an antagonist of said protein, can also be used for the fabrication of 10 drugs intended for the treatment or prevention of pathologies involving cerebral neuronal degeneration. The invention thus also relates to the pharmaceutical preparations comprising as an active ingredient, at least one of these proteins of the invention, possibly combined with a physiologically acceptable vehicle.

More specifically, the invention relates to a chemical or biological substance 15 that is capable of modifying the currents of an ionic channel and/or a hybrid channel according to the invention for the preparation of a drug capable of modulating the perception of acidity with regard to nociception as well as taste transduction in a human or animal subject.

Other characteristics and advantages of the invention will be seen in the 20 description below related to research activities that led to the demonstration and the characterization of the ASIC channel, and in which reference will be made to the annexed sequences and drawings in which:

Detailed Description of the Figures

Figure 1 represents the alignment of the sequences of the rat ASIC proteins (at top) and human ASIC proteins (at bottom) of sequences SEQ ID NO: 1 and SEQ ID NO: 2. Comparison of these sequences shows the absence of 14 5 amino acids at the beginning of the human coding phase compared to that of the rat.

Figure 2 represents a comparison of the protein sequence of the rASIC1A channel with the sequence of other ionic channels:

- ASIC2A (MDEG) [14], a mammalian cationic channel that is activated 10 by the mutations responsible for neuro-degenerations with the degenerines of *C. elegans*.
- FaNaCh [10], a peptide of a sodium channel of *Helix aspersa* that is activated by FMRFamide.
- The degenerine MEC-4 [12] of *C. elegans*.

15 In this figure, the residues that are identical or similar to those of ASIC are printed respectively in white on a black background and in black on a gray background. The supposed transmembranal regions (MI, MII) of rASIC1A are marked by black bars.

Figure 3 represents the phylogenetic tree of the proteins of the subunits 20 α NaCh, β NaCh, γ NaCh, δ NaCh of the amiloride-sensitive sodium channel and of the degenerines MEC-4, MEC-10 and DEG-1 of *C. elegans*.

Figure 4 represents the topology proposed for this latter family of ionic channels [30].

Figure 5 shows the biophysical properties of the proton-activated rASIC1A channel.

- a) the macroscopic inflowing currents recorded at -70 mV after rapid pH changes from pH 7.4 to pH 6.
- 5 b) the dose-response curve of the extracellular pH. The initial pH was 7.4 and the points represent the mean values from 6 tests. The insert in this Figure shows the typical responses at -70 mV.
- c) the Q-V relations of the outside-out patch with 140 mM of Na^+ (■) or of Li^+ (●) in the bath solution. Q is the charge transported during the acid pH 10 transition. The insert in this figure shows the typical responses in a medium containing Na^+ .
- d) the currents activated by the H^+ protons recorded at various potentials in an outside-out patch in a medium containing Na^+ .
- 15 e) the mean i-V relations measured from the outside-out patch with 140 mM of Na^+ (■), 140 mM of Li^+ (●) or 1.8 mM of Ca^{2+} (▲), as majority permeable ions in the external solutions; the inversion potentials were respectively 65 mV, 58 mV and -34 mV.
- f) the proton current through the rASIC1A channel. The relations between the current peak and the voltage were measured from an outside-out patch in 20 a solution of free Na^+ , free Ca^{2+} with pipettes containing a solution of free K^+ , at pH 4 (●) and at pH 3 (■), with (▲) representing the results obtained under the same conditions as (■) but with KCl in the pipette. The insert in this figure shows the typical responses under (▲) conditions.

Figure 6 shows the effect of Ca^{2+} and of amiloride on the rASIC1A current.

- a) the currents activated by the H^+ protons recorded at various membrane potentials from an outside-out patch with 1.8 mM of Ca^{2+} in a solution of free Na^+ ; the currents were inverted at -35 mV.
- b) the mean Q-V relations from an outside-out patch recorded in solutions of free Na^+ containing 1.8 mM of Ca^{2+} (○, inversion potential -34 mV) or 0.1 mM of Ca^{2+} (●, inversion potential -80 mV).
- c) the effect of the external Ca^{2+} on the macroscopic peak of inflowing current recorded at -70 mV and activated by a rapid pH change from pH 7.4 to pH 6. The insert in this Figure shows the typical responses. The points represent means values \pm se of 5 oocytes.
- d) the effect of amiloride on the currents activated by the H^+ protons recorded at 0 mV from an outside-out patch.
- e) the inhibition of the macroscopic current (induced by a pH change from pH 7.4 to pH 6) at -70 mV by amiloride and derivatives. The points represent the means values \pm se of 5 oocytes.

Figure 7 shows the tissue distribution of ASIC1A channel mRNA.

- a) Northern blot analysis of the mRNA expression of the hASIC1A channel in human tissues.
- b) In b: RT-PCR analysis of the mRNA expression of the rASIC1A channel in the rat brain and in the dorsal root ganglion (DRG). (+), (-) represent respectively the samples with or without reverse transcriptase. The agarose gel

sections were developed in 1% ethidium bromide. The arrows indicate the discounted size (657 pb) of the PCR product.

Figure 8 shows the *in situ* hybridization.

a,b) hybridization of 6 μ m sections of a dorsal root ganglion from a 3-year-old rat with the E probe marked with digoxigenin. In a: a low-lighting microphotograph (enlarge 30X). In b: a high-resolution image (enlarge 80X) of "a". One can see the intense marking of the small-diameter neurons (arrows). Similar results were also obtained with probes A, C and D.

c) the distribution of the rASIC1A channel mRNA in the brain of an adult rat analyzed by *in situ* hybridization with antisense oligonucleotide C. Identical results were obtained with oligonucleotide B. The colors indicate abundance (red: high expression; blue: not detectable). The abbreviations used in the Figure are as follows: Cer = cerebellum; Hip = hippocampus; OB = olfactory bulb; Cx = cortex.

Figure 9 shows the alignment of the deduced protein sequences of hASIC3 and rASIC3. Amino acids that are identical or similar in both sequences are printed white on black or black on grey background respectively. The two putative hydrophobic transmembrane domains are labelled with boxes. Sequences were aligned with the pileup program (Genetic Computer Group, Wisconsin).

Figure 10 shows the pH dependence and pharmacology of hASIC3. Proton-induced membrane currents were recorded from hASIC3-transfected COS cells using the whole-cell-suction-pipette technique.

a) pH dependence of the hASIC3 current. H^+ -gated currents were induced by decreasing the extracellular pH rapidly from pH 7.3 to the pH values indicated. The pH required for half maximal activation was pH 6.2 for the transient current and pH 4.3 for the sustained current.

5 b) H^+ induced hASIC3 currents depend on the resting pH. The extracellular pH was decreased rapidly from the indicated resting pH to pH 4. The currents in A and B are shown as the fraction of the saturation level of the Boltzmann fit. c) inhibition of hASIC3 by the diuretics amiloride and triamterene. In the dose-response curve for amiloride ($K_{0.5} = 15.9 \mu M$), currents are expressed 10 as fraction of the mean current in the absence of drug. Data points (o, transient current; I sustained current) represent the average $\pm SEM$ of at least 5 experiments. Macroscopic currents were recorded from cells clamped at -60 mV using the whole cell suction-pipette technique.

Figure 11 shows the selectivity and single channel properties of hASIC3.

15 a) voltage dependence of the transient and sustained whole cell current.

The transient current reverses at 37.6mV, the sustained current reverses at 10.1mV.

b) the voltage dependence of the unitary currents of spontaneously active channels at pH7.3 or of channels activated by a step to pH4. Slope 20 conductance between -10 and +40mV for both conditions is $15.0 \pm 0.6 pS$. $V_{rev} = 30.2 mV$. The Na^+ equilibrium potential is at 40.1mV. Examples of spontaneous channel activity at a resting pH of 7.3 (c) or activity evoked by a drop to pH4 (d). The channel-activity recorded at pH7.3 was inhibited by $100 \mu M$

amiloride (c). Single channel currents were recorded at -60mV from outside-out membrane patches excised from hASIC transfected COS cells.

Figure 12 shows the human chromosomal localization of the hASIC3 gene. The human ASIC3 gene is localized 6.4 cRad telomeric to the framework 5 marker AFMA082XC9 on chromosome 7 (lod score > 21). The position of hASIC3 relative to several microsatellites is shown in the right part of the Figure. The relative positions of the markers and their distances (in cRad) are the output of the RHMAPPER program. The microsatellites D7S676 and D7S642 are localized on band q35 of chromosome 7 (data from <http://www.ncbi.nlm.nih.gov>).
10 The cytogenic localization of those two markers is indicated with dashed lines.

Cloning the ASIC Channel

The conserved sequences of the family of ASIC ionic channels were used to prepare the following PCR primer sequences:

15 TTYCCIGCIRTIACIITNTGYAAY, and CAIARICCIAITGNCCNCCDAWRTC.

A bank of rat brain cDNA (Stratagene #936515) was hybridized with the PCR product of 1 kB of rat brain and the partial clones were isolated. The fifth extremity of the cDNA (202 bp) was isolated by PCR after ligation adapted to the double-strand cDNA.

20 *Electrophysiology*

0.25 ng of cRNA was injected into the *Xenopus laevis* oocytes and the recording microelectrodes for the imposed voltage and for the patch-clamp were installed two days after the injection. The bath solutions for the outside-

out patch recordings and the pipettes for the outside-out patch and total cells recordings contained: 140 mM KCl (or NMDG), 2 mM MgCl₂, 5 mM EGTA, 10 mM Hepes, pH 7.4 (with KOH). The pipettes for the outside-out patch recordings and the bath solutions for the outside-out patch and total cells

5 recordings contained: 140 mM NaCl (or LiCl or NMDGCl), 2 mM MgCl₂, 1.8 mM CaCl₂, 10 mM Hepes, pH 7.4 (adjusted with HCl, NaOH, LiOH or TMAOH). The rapid pH changes from the initial pH were obtained by perfusion with a bath solution adjusted to the pH indicated in the Figures. The intracellular acidification of the oocytes was implemented by injecting 50

10 ml of the internal solution at pH 2 or by perfusion and withdrawal of a bath medium containing 20 mM NH₄Cl. None of the recorded currents was contaminated by the Ca²⁺ current sensitive to the Cl⁻ of the *Xenopus* oocyte. The data were sampled at 2 kHz and filtered at 500 Hz for the analysis (Logiciel Biopatch).

15 *Northern blot analysis, RT-PCR and in-situ hybridization*

The Northern blot kit was obtained from Clontech Co. (Palo Alto, CA) and contained circa 2 µg of poly(A+) RNA per line. The blot was hybridized with a fragment of the partial human clone (corresponding to bases 270 to 764 of the rat clone) marked with ³²P at 65°C in 6xSSC. For the RT-PCR

20 analysis, 5 µg of rat brain total RNA and 3 µg of dorsal root ganglion were reverse transcribed and 1/30 of the sample was amplified by 30 PCR cycles with the following sequence primers:

ATTGCTCTTCCCCATCTCTAT, and TTCAAGGCCATACCTAAGT.

The negative controls were treated in an identical manner with the exception of the reverse transcriptase which was not added. The antisense oligonucleotides corresponding to base 70 to 114 (A), 215 to 248 (B), 1821 to 1859 (C), 1896 to 1940 (D) and the double-strand DNA corresponding to 5 base 1685 to 2672 were used for the *in-situ* hybridizations. The sections of adult rat brain were hybridized with oligonucleotides B or C the ends of which were marked with ^{32}P for one night at 37°C in 50% formamide, 2xSSC, then washed at ambient temperature in 1xSSC. The signal was eliminated by 500-times excess of unmarked oligonucleotides. The dorsal root ganglion 10 sections were hybridized with oligonucleotides A, C or D marked with digoxigenin (DIG)-dUTP and with probe E marked with DIG-dUTP by PCR. The marking of the probes, the preparation of the samples, the hybridization and the visualization of the DIG nucleic acids with alkaline phosphatase conjugated with anti-DIG antibodies were performed in accordance with the 15 supplier's protocols (Boehringer Mannheim).

Computer analysis

The sequence alignments and the phylogenetic tree (Kimura substitution, UPGMA option) were performed with the GCG program (Genetics Computer Group, Madison, WI).

20 *Identification of hASIC3*

Comparison of the rat DRASIC protein sequence with the database of expressed sequence tags (EST) identified two partial cDNA sequences from human total fetus (Genbank accession AA449579 and AA449322).

Both sequences originate from the same clone (IMAGE ID 785700) that we obtained from the UK HGMP RESOURCE CENTRE. Sequencing both strands using an Applied Biosystems automatic sequencer showed that the clone contains the entire coding sequence.

5 *Chromosomal localization*

The human ASIC3 gene was mapped by PCR on the Genebridge 4 Radiation Hybrid DNA panel with the primers CGATTGCAGTTCAGCATCTCT (sense) and ACCATTGGCAGCCGCACTT (antisense) at an annealing temperature of 65°C. The PCR products were 10 analyzed on 2% agarose gels. Samples were considered positive when a strong amplification of a 159 bp fragment was detected (Code 1), ambiguous when a faint amplification of this fragment was detected (Code 2) and negative when no amplification around 160 bp was visible (Code 0). The positive control (human genomic DNA) was positive and the negative control 15 (hamster genomic DNA) was negative. The following code sequence for the 83 radiation hybrids was obtained and entered into the RHMAPPER program on the Whithead Institute (<http://www-genome.wi.mit.edu>) with a Lod score cutoff of 21: 00000 00100 00001 00021 00100 12010 00000 12112 21000 00001 10120 00010 00102 11010 00010 00212 11011 00001 100.

20 *Expression in COS cells*

The vector containing the hASIC3 coding sequence was linearized with *Nos*I and blunt ended with T4 DNA polymerase. After inactivation of the T4 DNA polymerase, the hASIC3 coding sequence was excised with *Eco*RI

and subsequently subcloned into the *Eco*RI/*Sa*I (blunt) digested PCI expression vector (Promega). COS cells, at a density of 20.000 cells per 35 mm diameter petri dish, were transfected with a mix of CD8 and hASIC3-PCI (1:5) using the DEAE-Dextran method. Cells were used for electro-

5 physiological measurements one to three days after transfection.

Successfully transfected cells were recognised by their ability to fix CD8-antibody-coated beads [13].

Electrophysiology

Ion currents were recorded using either the whole cell or outside-out patch-clamp technique. The pipette solution contained (in mM): KCl 120, NaCl 30, MgCl₂ 2, EGTA 5, HEPES 10 (pH 7.2). The bath solution contained in mM: NaCl 140, KCl 5, MgCl₂ 2, CaCl₂ 2, HEPES 10 (pH 7.3). Changes in extracellular pH were induced by opening one out of six outlets of a microperfusion system in front of the cell or patch. Test solutions having a pH 15 of less than 6 were buffered with 10 mM MES rather than HEPES but were identical to the control solution in all other respects. Experiments were carried out at room temperature (20-24 °C).

Results

The 35 kb cDNA isolated from rat brain codes for a protein of 526 amino acids that exhibits, as shown in Figure 2, homologies with all of the 20 cloned members of the family of amiloride-sensitive degenerine sodium channels.

As shown in Figure 5, expression of the cRNA in the *Xenopus* oocytes induced an inflowing current activated by H⁺ protons. The biophysical and pharmacological properties of the rASIC1A channel are close to those described for the proton-activated cationic channels of sensory neurons [3, 5 15, 16]. Reduction of the extracellular pH below a pH of 6.9 activates a rapidly rising and desensitized inflowing current (Figure 5a and b). This channel is activated by extracellular protons since, as shown in Figure 5 (c and d), application of an acid on the extracellular surface of the outside-out patch activates the channel. Intracellular acidification of oocytes and 10 acidification of the intracellular surface of the outside-out patch does not activate the rASIC1A channel nor alter the rASIC1A current induced by the extracellular protons.

The analysis of curves I-V of Figure 5 (c and e) recorded with different extracellular cations shows that Na⁺ is the majority permeable ion (simple 15 conductance channel 14.3 pS). Like the proton-sensitive ionic channel of the sensory neurons [15, 16], the ASIC channel discriminates weakly between the cations (Figure 5c, e, f). In fact, the channel is also permeable to Li⁺, K⁺, Ca²⁺ and H⁺ with the ratios pNa⁺/pLi⁺ = 1.3 (Figure 5c, e), pNa⁺/pK⁺ = 13 (Figure 5c, e), pNa⁺/Ca²⁺ = 2.5 (Figure 5e) and pNa⁺/H⁺ = 0.8 (Figure 5f). 20 The permeability to Ca²⁺ of ASIC could be a voltage-independent entry path of Ca²⁺ into the cell. An inflowing current of Ca²⁺ into the cell via the ASIC channels can be detected in the absence of extracellular Na⁺ (Figure 6a, b). As indicated in Figure 5 (e), the unitary conductance for Ca²⁺ was 5.2 pS. In

the presence of 140 mM of extracellular Na^+ , augmentation of the concentrations of external Ca^{2+} diminished the amplitude of the current activated by the protons (Figure 6c), thereby demonstrating that Ca^{2+} inhibits the permeability to Na^+ . Blockage by external Ca^{2+} is characteristic of the 5 $I(\text{H}^+)$ of the sensory neurons [17]. The inflowing current activated by H^+ in the sensory neurons is inhibited by amiloride [18] and ethylisopropylamiloride (EIPA) [19]. As shown in Figure 6 (d, e), the rASIC1A channel exhibits the same pharmacology and is blocked in a reversible manner ($K_d = 10 \mu\text{M}$) by amiloride and its derivatives benzamil and EIPA.

10 In addition, the rASIC1A channel protein exhibits approximately 67% sequence homology with the degenerine ionic channel referred to as MDEG [14] or BNaCl [20], herein designated rASIC2. However, the electrophysiological properties of these two clones expressed in *Xenopus* oocytes are clearly different:

15 - As shown in Figure 5(a), the rASIC2 channel is not activated by the same pH changes as the rASIC1A channel.

- Substitution of the glycine residue in position 430 of rASIC2 by an acid-inhibiting amino acid such as valine or phenylalanine activates the channel [14], just as the mutation of alanine in position 704 of degenerine 20 MEC-4 causes neurodegeneration in *C. elegans* [12]. Identical mutations of rASIC1A (glycine in position 431 replaced by valine or phenylalanine) do not lead to activity and the mutants cannot be activated by protons.

Proton-activated cationic channels have been described not only in the sensory neurons but also in the neurons of the central nervous system [21]. The tissue distribution of the expression of the mRNA of the hASIC1A channel is in agreement with this observation. As shown in Figure 7a, a 4.3-5 kb transcript was detected in the brain by Northern blot analysis and the PT-PCR results presented in Figure 7b show that the dorsal root ganglion expresses the rASIC1A mRNA. Figure 8 (a, b) shows that rASIC1A mRNA is well expressed by the small neurons of the dorsal root ganglion, which supports the fact that ASIC is the rapidly desensitizing proton-activated 10 cationic channel described in the nociceptive sensory neurons. Whereas the presence of proton-activated cationic channels in the dorsal root ganglion is in agreement with their function of acidity detector in nociception, their role in the brain remains to be established. The results of *in-situ* hybridization in Figure 8c show a broad and heterogeneous expression of the rASIC1A 15 channel mRNA. The highest levels of expression were observed in the principal olfactory bulb, the cerebral cortex, the hippocampus, the habenula, the basolateral amygdaloid nucleus and the cerebellum. The synaptic activity accompanies extracellular pH changes [22, 23] and the rapid localized pH changes in or close to the synaptic cleft are noticeably more saturated and 20 stronger than the reported macroscopic fluctuations in the pH.

The proton-activated cationic channels are the only known ionic channels that are directly activated by a change in pH and it was envisaged that the extracellular fluctuations in pH played a neuromodulator role [23].

The expression of cationic channels in the brain supports in addition the hypothesis that the pH fluctuations are not solely a neuronal activation by a product, but even more a communications pathway in the central nervous system.

5 In addition to the rapidly inactivated proton-activated cationic channels, the presence has been reported in the sensory neurons of proton-activated cationic channels exhibiting slower kinetics [4, 24]. The proton-activated cationic channels probably form, like other cationic channels activated by a ligand [25, 26], a family of cationic channels in which different 10 subunits or combinations of subunits constitute channels with diverse pharmacological and biophysical properties.

The sensation of acidity is not uniquely implicated in nociception but is also associated with the transduction of taste [2]. Acid stimulations activate the proton-activated cationic channels in the taste cells [2, 27] and amiloride 15 inhibits the perception of acid taste [2]. Also, the physiological as well as pharmacological data indicate that rASIC1A and other members of this family are implicated in the transduction of taste. It is, in fact, especially surprising that the same class of ionic channels is associated with different facets of sensory perception:
20 - the amiloride-sensitive sodium channels are associated with the transduction of salty taste [2].

- the degenerines of *C. elegans* are implicated in mechanotransduction and have been proposed as forming the mechanosensitive ionic channels [28, 29].

- the ASIC family of channels are implicated in nociception and the
- 5 transduction of acid taste.

Comparison of the rASIC3 sequence with the database of expressed sequence tags identified a novel human member of this ion channel family.

This novel clone from a total human embryo library codes for a protein of 533 amino acids that shares the closest homology (84% identity, 87% homology)

- 10 with rASIC3 (Fig. 9). The cloning of a nearly identical cDNA from human testis (hTNaC1), although without functional expression, was reported recently [14].

Expression of the novel hASIC3 clone in COS cells induced a H⁺-gated cation current with kinetics very similar to that of rASIC3. When the pH is

- 15 decreased rapidly from pH 7.3 to pH 5, a biphasic current is observed. A rapidly inactivating component is followed by a sustained current (Fig. 10A). These very peculiar kinetics that are also found with the rASIC3 [9] channel together with the sequence homology (84% amino acid, 82% nucleic acid identity) with rASIC3 suggest that this novel clone is the human ASIC3. We
- 20 therefore call it hASIC3 (human Acid Sensing Ion Channel 3).

The pH dependence of the transient hASIC3 current ($\text{pH}_{0.5}=6.2$, Fig. 10A) is almost identical to that reported for rASIC3 ($\text{pH}_{0.5}=6.5$) [9]. However, the pH dependencies of the sustained rASIC3 and hASIC3 currents are

clearly different. While rASIC3 requires very acidic pH values (<pH 4.5) [9] for activation of the sustained current, the sustained hASIC3 current starts to activate when the extracellular pH decreases to below pH 6 and reaches half-maximal activity at pH 4.3 (Fig. 10A). The channel activity of hASIC3

5 depends, just as that of the rASIC3 channel, on the resting pH (Fig. 10B). The maximal activity of the transient hASIC3 current was observed when the resting pH was above pH 8, indicating that a fraction of the transiently activating H⁺-gated cation channels are inactivated at physiological pH. Half-maximal activation of the transient current was observed at pH 7.5, a slightly

10 more alkaline pH than that reported for the rASIC3 clone (pH 6.5) [9]. When the resting pH was below pH 7, only activation of the sustained current could be observed after acidification of the bath medium (Fig. 10B). The sustained hASIC3 current can, just as the sustained rASIC3 channel, still be activated when the initial pH is quite acidic (pH5) (Fig. 10B).

15 All members of the ASIC family cloned so far are sensitive to the diuretic amiloride. The hASIC3 channel is no exception. The effect of amiloride on the hASIC3 current is similar to that reported for rASIC3 [9]. The transient current is inhibited by amiloride ($K_D=15.9 \mu M$; Fig. 2C) as well as by triamterene (Fig. 10C), while the sustained hASIC3 current is virtually not

20 affected by those diuretics.

The transient hASIC3 current reverses at 37.6 mV, close to the Na⁺ reversal potential, indicating a high selectivity for Na⁺ vs K⁺ (Fig. 11A). Conversely, the sustained current discriminates much less between Na⁺ and

K^+ (selectivity ratio $gNa^+/gK^+ = 1.62$) as it reverses at 10.1 mV (Fig. 11A). The low selectivity for Na^+ vs K^+ of the sustained hASIC3 current clearly distinguishes the hASIC3 channel from the rASIC3 channel which is highly selective for Na^+ [9].

5 Proton-induced unitary currents were recorded from excised outside-out patches (Fig. 11B-D). In a narrow pH window around pH 7.3, spontaneous channel activity can be observed (Fig. 11C) that disappears upon an increase in pH to 8.0, a decrease in pH to 6.0 (not shown) or in the presence of 100 μ M amiloride (Fig. 11C). This basal current is mainly carried
10 by Na^+ , since it reverses at 30.2 mV (Fig. 11B). When the pH on the extracellular face of an outside-out patch is decreased from pH 7.3 to pH 4, unitary currents are induced (Fig. 11D) that reverse at the same membrane potential as the spontaneously active channel (Fig. 11B). The unitary conductance of the hASIC3 channel for Na^+ is 15 ± 0.6 pS, close to that
15 reported for rat ASIC3 (12.6 pS) [9]. While the sustained non-selective H^+ -activated hASIC3 current could be easily detected in whole cell recordings, no sustained or non-selective current could be recorded on outside-out patches. One possible explanation is, that soluble factors might be necessary that are lost during excision of the patch.

20 The human chromosomal localization of the hASIC3 gene was determined by PCR on a human-hamster radiation hybrid DNA panel. The hASIC3 gene is localised on the human chromosome 7q35, 6.4 cRad telomeric from the microsatellite AFMA082XC9 (Lod score > 21). To our

knowledge, no hereditary diseases with symptoms that are consistent with an altered function of a H⁺-gated cation channel were mapped to this region of the human genome.

The hASIC3 channel subunit forms a sustained H⁺-gated cation channel that has properties similar to those reported for the rASIC3 channel. However, very important differences exist. Most importantly, the sustained hASIC3 current requires less acidic pH for activation than rASIC3 [9]. In this respect the properties of the hASIC3 channel match better the physiological and electrophysiological data from sensory neurones than those of rASIC3.

Subcutaneous perfusion of human volunteers with acidic buffer causes pain. At pH 5.2, the pain was rated 20% on a scale ranging from 0 to 100% (unbearable pain) [2]. Furthermore, a subpopulation of polymodal C-fibres in rat nerve-skin preparations can be excited by acidic pH [4]. The threshold for activation lies between pH 6.9 and pH 6.1, maximal stimulation is reached at pH 5.2. The endogenous H⁺-gated cation channel recorded in rat sensory neurones starts to activate below pH 6.6 [5]. The pH dependence of the sustained hASIC3 current matches closely those physiological data, while rASIC3 has a pH dependence that is shifted two pH units towards more acidic pH values [9]. One possible explanation for the differences between physiological data and the pH dependence of the sustained ASIC3 channel (especially the rASIC3) might be the participation of as yet unknown subunits in the formation of the native channel. Heteromultimeric assembly was previously demonstrated for the rASIC3 channel [9]. rASIC3 can associate

with rASIC2b resulting in an altered selectivity of the channel. While rASIC3 is completely Na^+ -selective, the sustained current of the heteromultimeric rASIC3/rASIC2b channel does not discriminate between Na^+ and K^+ . The H^+ -gated cation channel recorded in rat sensory neurones does not discriminate 5 between Na^+ and K^+ either [5], suggesting that both rASIC3 and rASIC2b participate in the formation of this ion channel in rat sensory neurons. In contrast with the rASIC3 channel, hASIC3 does not require coexpression of other subunits to generate a non-selective sustained current. The ion selectivity of sustained human H^+ -gated cation channels is not known yet. A 10 more detailed electrophysiological characterization of human sustained H^+ -gated cation channels will be necessary to allow a comparison of the properties of the native channel with those of the hASIC3 channel.

List of Sequences

INFORMATION CONCERNING SEQ ID NO: 1

15 i) CHARACTERISTIC OF THE SEQUENCE:
 A) LENGTH: 3562 base pairs
 B) TYPE: nucleic acid
 C) NUMBER OF STRANDS: double
 D) CONFIGURATION: linear
20 ii) TYPE OF MOLECULE: DNA
 vi) ORIGIN: rat
 ix) CHARACTERISTIC
 A) NAME/KEY: ASIC

B) LOCALIZATION: 123 .. 1700

xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 1:

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5 **INFORMATION CONCERNING SEQ ID NO: 2**

i) CHARACTERISTIC OF THE SEQUENCE:

A) LENGTH: 1620 base pairs

B) TYPE: nucleic acid

C) NUMBER OF STRANDS: double

10 D) CONFIGURATION: linear

ii) TYPE OF MOLECULE: DNA

vi) ORIGIN: human

ix) CHARACTERISTIC

A) NAME/KEY: ASIC

15 B) LOCALIZATION: 1 .. 1542

xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 2:

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20 **INFORMATION CONCERNING SEQ ID NO: 3**

i) CHARACTERISTIC OF THE SEQUENCE:

A) LENGTH: 1666 base pairs

B) TYPE: nucleic acid

C) NUMBER OF STRANDS: double

- D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC

5 A) NAME/KEY: MDEG

- B) LOCALIZATION: 127 .. 1663
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 3:

Top of page 34 =

10 **INFORMATION CONCERNING SEQ ID NO: 4**

- i) CHARACTERISTIC OF THE SEQUENCE:
- A) LENGTH: 3647 base pairs
- B) TYPE: nucleic acid
- C) NUMBER OF STRANDS: double

15 D) CONFIGURATION: linear

- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- ix) CHARACTERISTIC

A) NAME/KEY: ASIC1B

20 B) LOCALIZATION: 109 .. 1785

- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 4:

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INFORMATION CONCERNING SEQ ID NO: 5

i) CHARACTERISTIC OF THE SEQUENCE:

5 A) LENGTH: 1602 base pairs

 B) TYPE: nucleic acid

 C) NUMBER OF STRANDS: double

 D) CONFIGURATION: linear

ii) TYPE OF MOLECULE: DNA

 vi) ORIGIN: rat

10 ix) CHARACTERISTIC

 A) NAME/KEY: ASIC3

 B) LOCALIZATION: 1 .. 1602

 xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 5:

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15 **INFORMATION CONCERNING SEQ ID NO: 6**

i) CHARACTERISTIC OF THE SEQUENCE:

 A) LENGTH: 1948 base pairs

 B) TYPE: nucleic acid

 C) NUMBER OF STRANDS: double

20 D) CONFIGURATION: linear

 ii) TYPE OF MOLECULE: DNA

 vi) ORIGIN: rat

 ix) CHARACTERISTIC

- A) NAME/KEY: ASIC2B
- B) LOCALIZATION: 16 .. 1707
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 6:

5

INFORMATION CONCERNING SEQ ID NO: 7

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 1736 base pairs
 - B) TYPE: nucleic acid
 - C) NUMBER OF STRANDS: double
 - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC:
 - A) NAME/KEY: ASIC3
 - B) LOCALIZATION: 18 .. 1611
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 7:

INFORMATION CONCERNING SEQ ID NO: 8

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 531
 - B) TYPE: protein
 - C) NUMBER OF STRANDS: single
 - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: protein

- vi) ORIGIN: human
- ix) CHARACTERISTIC
- A) NAME/KEY: hASIC3
- B) LOCALIZATION: 1 - 531

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We claim:

1. An isolated and purified nucleic acid molecule encoding the human cation transport protein ASIC3.
2. The sequence of claim 1 which maps to chromosome 7q35.
- 5 3. The sequence of claim 1 represented by SEQ ID No. 7.
4. An isolated and purified human cation transport protein represented by SEQ ID No. 8.
5. The protein of claim 4 which comprises a proton gated cation channel.
- 10 6. The protein of claim 5 wherein the transport channel activity is dependent upon resting pH.
7. The protein of claim 5 which also exhibits biphasic desensitization kinetics.
8. The protein of claim 4 which is sensitive to amiloride.
- 15 9. A human cation transport channel comprising at least one molecule of ASIC3 and at least one molecule of a second human proton-gated cation channel.
10. The cation transport channel of claim 9 wherein the second cation transport protein is selected from the group consisting of hASIC2A, hASIC2B,
- 20 hASIC1A and hASIC1B.
11. A cloning vehicle comprising the sequence of claim 1.

12. The cloning vehicle of claim 11 which also comprises a sequence encoding a second cation transport protein selected from the group consisting of hASIC2A, hASIC2B, hASIC1A and hASIC1B.
13. A transformed cell containing the cloning vehicle of claim 11.
- 5 14. The transformed cell of claim 13 which expresses hASIC3.
15. A transformed cell containing the cloning vehicle of claim 12.
16. The transformed cell of claim 15 which expresses a human cation transport channel comprising at least one molecule of ASIC3 and at least one molecule of a second cation transport protein selected from the group consisting of hASIC2A, hASIC2B, hASIC1A and hASIC1B.
- 10 17. A method of screening for substances capable of modulating the activity of cation transport channels comprised of hASIC3, comprising contacting pre-selected amounts of the substance to be tested with cells expressing said cation transport channel, measuring the effects of the substance on the transport functions of the cation transport channel, and identifying the substance that has a positive or negative effect on potassium channel activity .
- 15 18. A substance, identified by the method of claim 20 that is capable of positively or negatively influencing the transport functions of a cation transport channel.
- 20

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Met Glu Leu Lys Thr Glu Glu Glu Val Gly Val Gln Pro Val Ser Ile
Pro Val Ser Ile

Gln Ala Phe Ala Ser Ser Ser Thr Leu His Gly Leu Ala His Ile Phe Ser Tyr
Gln Ala Phe Ala Ser Ser Thr Leu His Gly Met Ala His Ile Phe Ser Tyr

Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala Leu Cys Phe Leu Gly Ser Leu
Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala Leu Cys Phe Leu Gly Ser Leu

Ala Val Leu Leu Cys Val Cys Thr Glu Arg Val Gln Tyr Tyr Phe Cys Tyr His
Ala Val Leu Leu Cys Val Cys Thr Glu Arg Val Gln Tyr Tyr Phe His Tyr His

His Val Thr Lys Leu Asp Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val
His Val Thr Lys Leu Asp Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val

Thr Leu Cys Asn Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu
Thr Leu Cys Asn Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu

Tyr His Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp
Tyr His Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp

Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn Phe
Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn Phe

Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp Arg Ala Gly
Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp Arg Ala Gly

His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg Gly Glu Ala Cys Ser
His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg Gly Glu Val Cys Ser

Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr Gly Lys Cys Tyr Thr Phe Asn
Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr Gly Lys Cys Tyr Thr Phe Asn

Ser Gly Gln Asp Gly Arg Pro Arg Leu Lys Thr Met Lys Gly Gly Thr Gly Asn
Ser Gly Arg Asn Gly Arg Pro Arg Leu Lys Thr Met Lys Gly Gly Thr Gly Asn

Gly Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly
Gly Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly

Glu Thr Asp Glu Thr Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln
Glu Thr Asp Glu Thr Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln

Asp Glu Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln
Asp Glu Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln

Figure 1

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Thr Phe Val Ser Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp Gly
Thr Phe Val Ala Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Pro Pro Trp Gly
Thr Cys Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr Ser
Thr Cys Lys Ala Val Thr Met Asp Ser Asp Leu Asp Phe Phe Asp Ser Tyr Ser
Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn
Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn
Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu Gln Tyr
Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu Gln Tyr
Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys Asp Gln Glu Tyr
Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys Asp Gln Glu Tyr
Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met
Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met
Val Lys Ile Pro Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys
Val Lys Ile Pro Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys
Ser Glu Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val
Ser Glu Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val
Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu
Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu
Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val
Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val
Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu Cys Arg Arg
Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Lys Leu Cys Arg Arg
Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val Ala Leu
Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val Ala Leu
Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu Arg Gly His Pro
Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu Arg Gly His Pro
Ala Gly Met Thr Tyr Ala Ala Asn Ile Leu Pro His His Pro Ala Arg Gly Thr
Ala Gly Met Thr Tyr Ala Ala Asn Ile Val Pro His His Pro Ala Arg Gly Thr
Phe Glu Asp Phe Thr Cys
Phe Glu Asp Phe Thr Cys

Fig 1 (continued)

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FaNaCh	1	M S W M Q N L K N Y Q H L R D P S E Y M S Q V Y G D P L A Y Q E N T K F V T E R E Y Y E D F G Y G E C F N S S E E V	M K Y T S A A T K P G V F P E H H Q H A M M R N R Y H P H H C N Y
MEC-4			
ASIC	1	[REDACTED]	[REDACTED]
MDEG	1	D L K T E E E V G G Q P V S I Q A F A S S I S T L H G A H I F S Y E R L S T K R A I L W A H C F I G S L	
FaNaCh	34	D L K E S P S E . G S Q P S S I Q A F A S S I S T L H G A H I F S Y E R L S T K R A I L W A H C F I G S L	
MEC-4	61	Q C E L I T G E F D P K L L P Y D K R L A W H F K E E C Y K T S A H G I P . M I G E A P N V Y R A V W V W L F I G C M	
ASIC	55	A Y L L C V C C I E R V Q Y Y F C Y H H V T K I D E V A A S Q U L T F P A V T L C N L N E F R S Q Y S K N D L Y H A G	
MDEG	54	C Y L L V E S S E R V S Y Y F S Y Q H V T K I D E V V A Q E V P A V T L C N L N G E F S R T I N D L Y H A G	
FaNaCh	79	T A A T L Q L S S L V R K Y L Q Q V V E L S E I K D S M P M Q P S V S I C N I E P I S L R T R S R M Y F N N	
MEC-4	120	I M L Y L N A Q S V I D K Y N R N E K . V D I Q A L K F D T A . P F P A I T L C N L N P Y K A S L A T S . V D L V K R T L S	
ASIC	113 E L L A L N R Y E I P D I O N R Y E K Q L E I L Q D K A N F R	
MDEG	112 E S Q N L B T W L R F L Q K F R F E Q D S F M N S S R A F Y E N L	
FaNaCh	135 E I W M Y L Q G G T P T E D P N F L E A M G F Q D S A T D E V A I V	
MEC-4	359	[REDACTED]	
ASIC	146	S F K P K P F N N R E F Y D R A G H D D R D M L S C H F R G E A C S A D F K V V F T H Y G K C Y T F	
MDEG	145	H Y K P K Q F E S M L E F L H R Y G H D L K D M L Y C K F G G E C G H Q D F T E V F T I K Y G K C Y M	
FaNaCh	168	G Q D A K K L S G Q D A K K L S H C R F N R E L C H V S N F S T F D G N Y F N C F T F	
MEC-4	419	T K A K E N I M F A M A T I S M A Q D R E R L S T K R E L I V . H K C S F N G K A C D I E A D F L T H I D P V F G S C F T F	
ASIC	198	N S G Q D G R P R R L K T M K G G T G N G L E I M L D I Q Q D E Y L P V W G E T D E T S F E A G L K V Q I H S Q D	
MDEG	197	N S G E D G K P L L T T W K G G T G N G L E I M L D I Q Q D E Y L P V W G E T D E T S F E A G V K V Q I H S Q S	
FaNaCh	212	N S G Q H N R T V N L T S I R A G P M Y G L R M V V N A S D Y W P T I E A T G V R L T I H D K E	
MEC-4	479	[REDACTED]	
ASIC	254	E P P F I D D I L G F G V A P G F Q T F V S C Q E Q R L I Y L P S P W G T C N A V T M D S D F F D S Y S I T A C R I	
MDEG	253	E P P F I Q Q L G F G V A P G F Q T E V S T Q E Q R L I Y L P P P W G E C R B S E M G L D E F P V Y S I T A C R I	
FaNaCh	268	S M P S P V D H G T D A P P G Y S S S V Y S L K A I L H T R L P Y P G N C T N D M L L N G I K Q Y K I Y S N Y E Y S V E G C Y R	
MEC-4	526	D F P F S P D T F G Y S A P T G Y V S S F G L R K M S R L P A Y G R C V P D G K T S D Y I Y S N Y E Y S V E G C Y R	

Figure 2

ASIC	311	DC ETRY VEN C NCRM V HMP GDA P	CT P EQ
MDEG	310	DC ETRY VEN C NCRM V HMP GDA P	CT P EQ
FaNaCh	325	LCK KORL I KRC GCK SSSA P	CT P EQ
MEC-4	586	SCF QQL KEC RCG DP RFF P V PEG	CT P EQ
ASIC	340	YKECA KPAL DFL YEKD	CMP C NLTRY GKE L S M V K I P
MDEG	339	HKECA EPAL GLL AEKD	SNYC C RT PC NLTRY N KEL S M V K I P
FaNaCh	385	YLACE EER E QK N L NNDRT Y	QPC SET SY L K S M S SY W P L E F Y Q L S A V E R F F K Q E R
MEC-4	622	CL A RM N D L G G L H G . . .	S F R C R C Q Q P C G Q Y S V T Y S P A K W P S L S L Q I Q L G . . .
ASIC	381	SK A S A K Y L A K K F N K S E Q	Y I G E N I
MDEG	380	SK T S A K Y L E K K F N K S E K	Y I S E N I
FaNaCh	445	QA G Q N H F M K T Y E Y L E K L A H P S I K	H L A R N D S H M D D I L S K S Y S L S E K E M A K E A S D L I R N E G
MEC-4	670	...	SC N G T A V E C N K H Y K E N G
M II			
ASIC	404	L VLD I F F E V L N Y E T I E Q K K A Y E I A	L T M L E L F D Y A Y E N S K H E L
MDEG	403	L VLD I F F E A L N Y E T I E Q K K A Y E I A	L L G D I G G Q W G L F I G A S L T I L E L F D Y Y E L I K E E L
FaNaCh	505	L R L I F F E D L S Y V E Y R Q L P A Y G I A D L F D I G G T G L W G I S L T I M E L I F T R L T G L V F	L D F V N L L I D F G Q E L G L W G I S F L T C C E F V F L F L E T A Y M S A
MEC-4	687	A M V E Y F I E Q L N M E M E T T S E A Y G F V N L L	...
ASIC	464	C R R G K C Q K E A K R S S A D K G N L S L D D V K R H N P C S	R G H P A G Y Y A N N L P H H P A R G T F E D F C
MDEG	463	L D L L G K E E E G S H D E N S	Y A N N L P L Q T A L G T E E P C
FaNaCh	585	N S E K G L P G P T T V N N N G S N N H S Q S T S Q H Q L Y G Y M S D S H Y S D S A G S V F D F R R G V E S P
MEC-4	747	E H N Y S L Y K K K A E K A K V A S G S F

Fig. 2 (continued)

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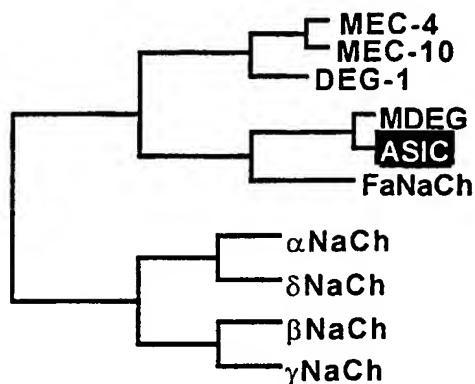


Figure 3

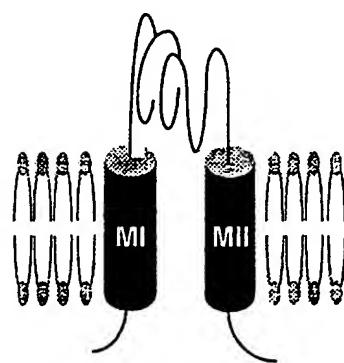
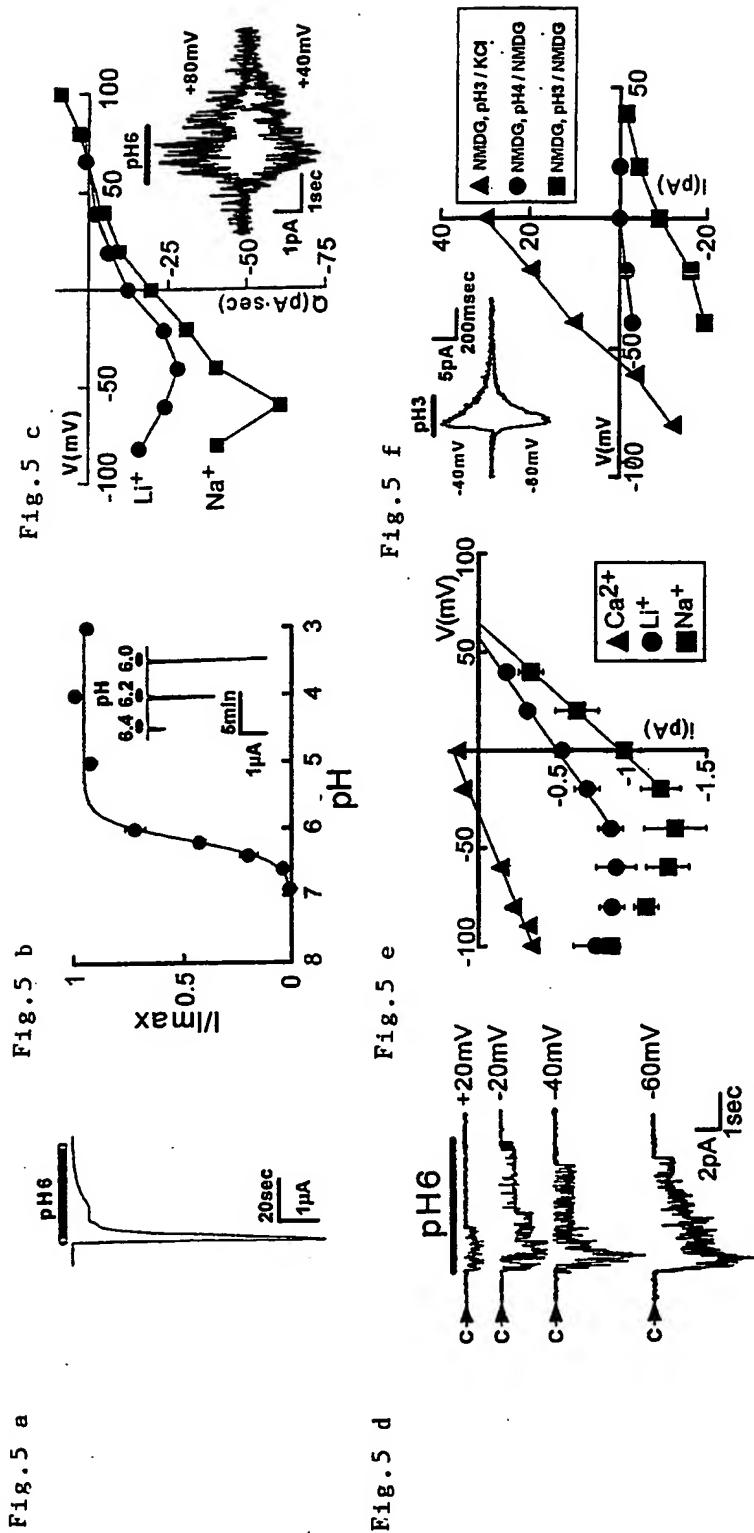


Figure 4

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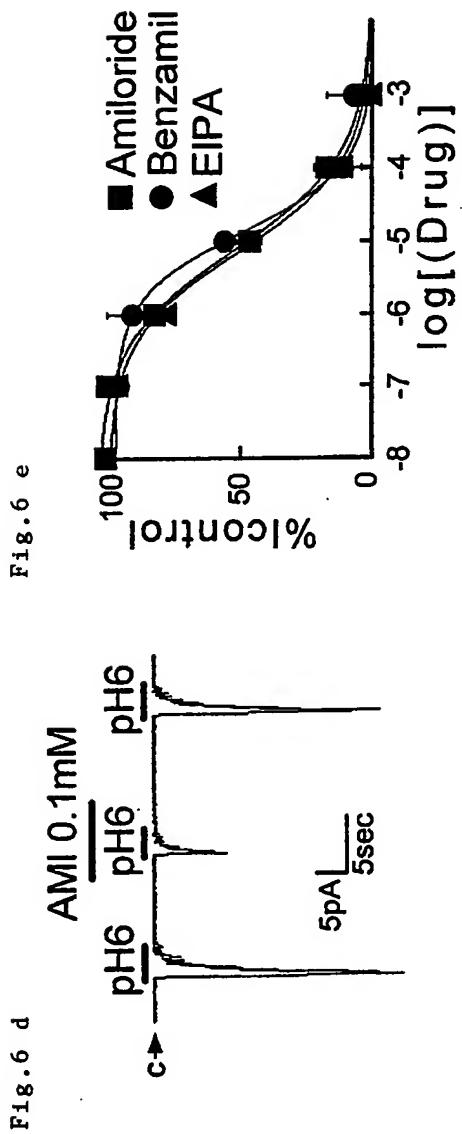
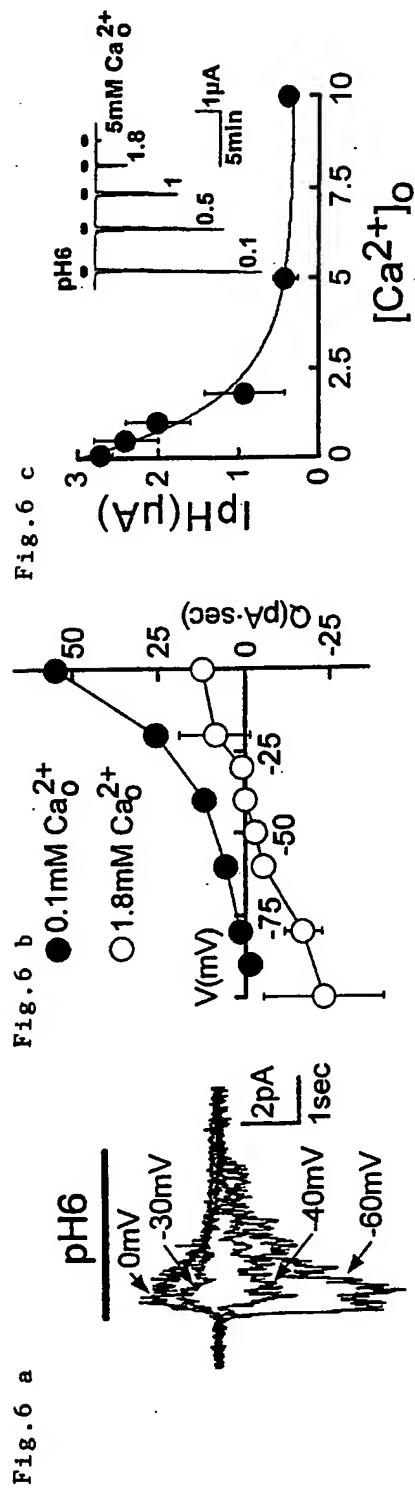
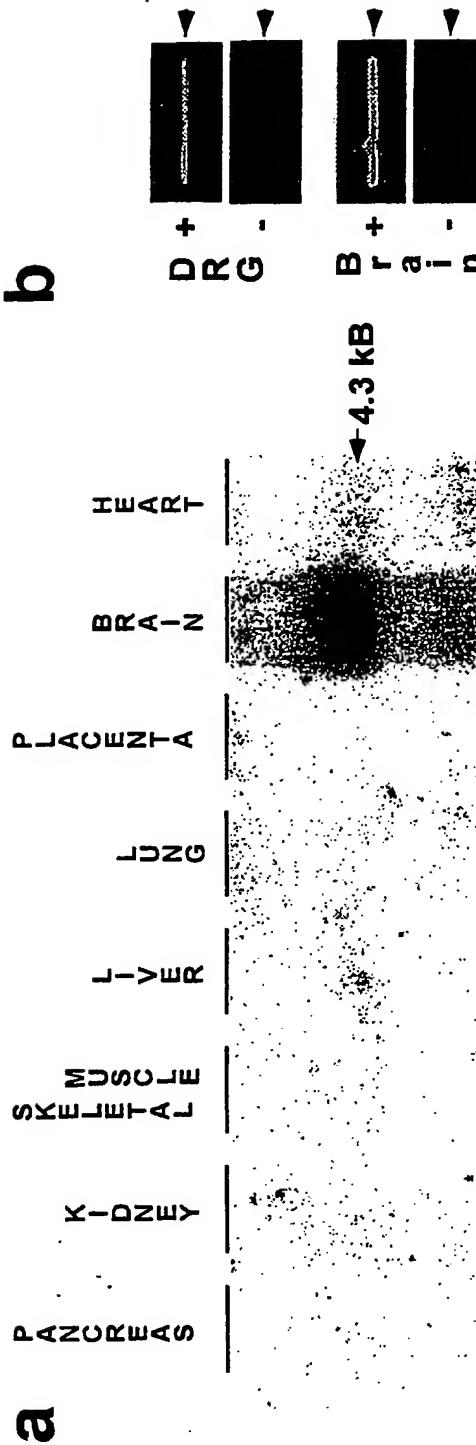


Fig. 7

**a**

a

Fig. 8 a

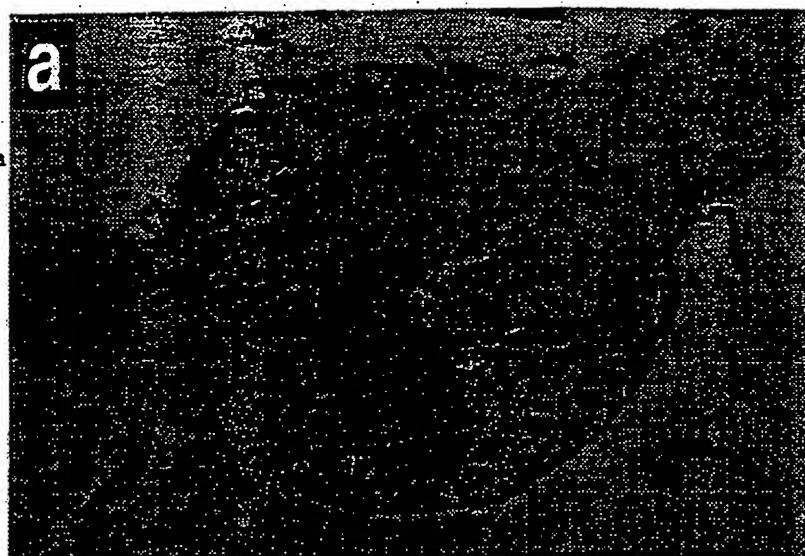
**b**

Fig. 8 b

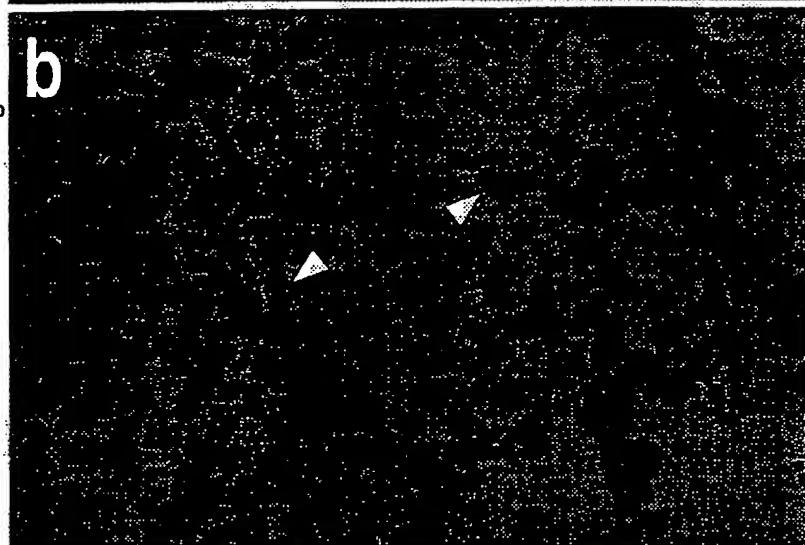
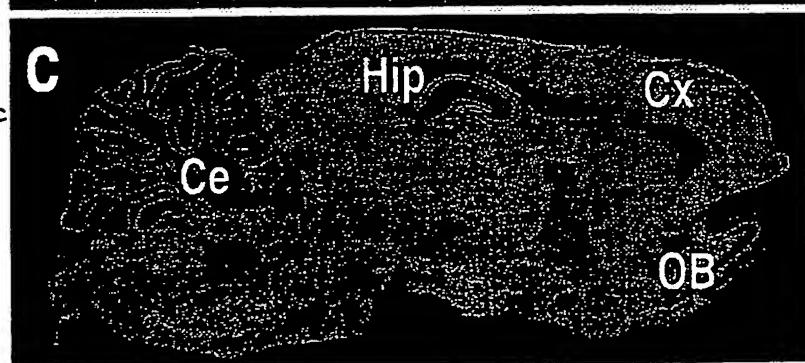
**c**

Fig. 8 c



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1 MKPTSGPEEA-RRPASDIRVFASSCWMHGLGHYFGPGESESLRGNWAAVAVLSVATEFLYQ
hASIC3 1 MKPTSGLEEAQRRQASDIRVFASSCWMHGLGHYFGPGESESLRGNWAAVAVLSVATEFLYQ
rASIC3 60 VAERVRYYREFHHQTALDERESHRLIEPAVTLCNINPLRRSRLLTPNDLHWAGSALLGLDP
rASIC3 61 VAERVRYYGEFHHKTFLDERESHQLTEPAVTLCNINPLRRSRLLTPNDLHWAGSALLGLDP
hASIC3 120 AEHAAFLRALGRPPAPPGEFMSPTFDMAQLYARAGHSDDMLLDCRFRGQQPCGPENETTI
rASIC3 121 AEHAAFLRALGQPPAPPGEFMSPTFDMAQLYARAGHSLEDMLLDCRYRGQQPCGPENETTI
hASIC3 180 FTRMGKCYTFNSGADGAELLTTGGMNGLDIMLDVQQEYLPVWRDNEETPFEVGIRV
rASIC3 181 FTRMGQCYTFNSGAHGAELLTTGGAGNGLIMLDVQQEYLPVWRDNEETPFEVGIRV
hASIC3 240 QIHQSPEPFIDQLGVSPGQYQTFVSCQQQLSFLPPPWGDCSASLNPXPEPEPSDP
rASIC3 241 QIHQSPEPAIDQLGPGAAQPGHQTFVSCQQQLSFLPPPWGDCNTPASLNPXPEPEPSDP
hASIC3 299 LGSPSPSPSPYTMGCRACETRYVARKCGRMVYMPG'DVPVCSQQYKNCAPAI
rASIC3 301 LGSPSPSPSPYSEIGCRACESEYVARKCGRMVYMPG'DVPVCSQQYKDCASPALDAM
hASIC3 359 LRKDISCACPNPCASTRYAKELSMVRIPSRAARFLARKLNREAYIAENVALD
rASIC3 361 LRKDTACVCPNPCASTRYAKELSMVRIPSRAARFLARKLNREAYIAENVALD
hASIC3 419 NYETVEOKKAYEMSEILGDIGGQMGLEIGASLITTLEIDLYLCEVFRDKVLYFWNRQHS
rASIC3 421 NYETVEOKKAYEMSEILGDIGGQMGLEIGASLITTLEIDLYLCEVFRDKVLYFWNRQHS
hASIC3 479 QRISSNTLQEGLSHRTQVPHLSLGPRTPPCAVTKTLLSASHRTCYLVTQI
rASIC3 481 QRISSNTLQEGLSHRTQVPHLSLGPRTPPCAVTKTLLSASHRTCYLVTQI

Figure 9

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Fig. 9 continued

Human ASIC3 sequence

10	30	50
ACGACGGGTTCTGGCCATGAAGCCCACCTCAGGCCAGAGGAGGCCGGCCAGCCT		
M K P T S G P E E A R R P A S		
70	90	110
CGGACATCCCGTGTTCGCCAGCAACTGCTCGATGCACGGCTGGCCACGTCTCGGGC		
D I R V F A S N C S M H G L G H V F G P		
130	150	170
CAGGCAGCCTGAGCCTGCGCCGGGGATGTGGGCAGCGGCCGTGGTCCTGTCAGTGGCCA		
G S L S L R R G M W A A A V V L S V A T		
190	210	230
CCTTCCTCTACCAGGTGGCTGAGAGGGTGCCTACTACAGGGAGTTCCACCACAGACTG		
F L Y Q V A E R V R Y Y R E F H H Q T A		
250	270	290
CCCTGGATGAGCGAGAAAGCCACCGGCTCATCTCCGGCTGTCACCCGTGCAACATCA		
L D E R E S H R L I F P A V T L C N I N		
310	330	350
ACCCACTGGCCGCTGGCCTAACGCCAACGACCTGCACTGGCTGGCTGCGCTGC		
P L R R S R L T P N D L H W A G S A L L		
370	390	410
TGGGCCTGGATCCCGCAGAGCACGCCCTTCCCTGCGCCCTGGCCGGCCCCCTGCAC		
G L D P A E H A A F L R A L G R P P A P		

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Fig.9 continued

430	450	470
CGCCCGGCTTCATGCCAGTCCCACCTTGACATGGCGCAACTCTATGCCCGTGTGGC		
P G F M P S P T F D M A Q L Y A R A G H		
490	510	530
ACTCCCTGGATGACATGCTGCTGGACTGTCGCTTCCGTGGCCAACCTTGTGGCCTGAGA		
S L D D M L L D C R F R G Q P C G P E N		
550	570	590
ACTTCACCACGATCTCACCCGGATGGGAAAGTGCTACACATTAACTCTGGCGCTGATG		
F T T I F T R M G K C Y T F N S G A D G		
610	630	650
GGGCAGAGCTGCTCACCACTACTAGGGGTGGCATGGCAATGGGCTGGACATCATGCTGG		
A E L L T T T R G G M G N G L D I M L D		
670	690	710
ACGTGCAGCAGGAGGAATATCTACCTGTGTGGAGGGACAATGAGGAGACCCGTTGAGG		
V Q Q E E Y L P V W R D N E E T P F E V		
730	750	770
TGGGGATCCGAGTGCAGATCCACAGCCAGGAGGGAGCCGCCATCATCGATCAGCTGGC		
G I R V Q I H S Q E E P P I I D Q L G L		
790	810	830
TGGGGGTGTCCCCGGCTACCAGACCTTGTGCTTGCCAGCAGCAGCTGAGCTTCC		
G V S P G Y Q T F V S C Q Q Q Q L S F L		
850	870	890
TGCCACCGCCCTGGGCGATTGCAGTTCACTCTGAACCCAACTATGAGCCAGAGC		

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Fig. 9 continued

P P P W G D C S S A S L N P N Y E P E P

910 930 950

CCTCTGATCCCCTAGGCTCCCCAGCCCCAGCCCTCCCTATACCCTTATGGGTT

S D P L G S P S P S P S P Y T L M G C

970 990 1010

GTCGCCTGGCCTGCGAAACCGCTACGTGGCTCGGAAGTGGCGCTGCCAATGGTGTACA

R L A C E T R Y V A R K C G C R M V Y M

1030 1050 1070

TGCCAGGCGACGTGCCAGTGTGCAGCCCCAGCAGTACAAGAACTGTGCCACCGGCCA

P G D V P V C S P Q Q Y K N C A H P A I

1090 1110 1130

TAGATGCCATGCTTCGCAAGGACTCGTGGCCTGCCAACCGTGCAGCACCGCT

D A M L R K D S C A C P N P C A S T R Y

1150 1170 1190

ACGCCAAGGAGCTCTCCATGGTGGAGATCCCGAGCCGCGCCGCGCGCTTCTGGCC

A K E L S M V R I P S R A A A R F L A R

1210 1230 1250

GGAAGCTAACCGCAGCGAGGCCTACATCGCGAGAACGTGCTGGCCCTGGACATCTTCT

K L N R S E A Y I A E N V L A L D I F F

1270 1290 1310

TTGAGGCCCTCAACTATGAGACCGTGGAGCAGAAGAAGGCCTATGAGATGTCAGAGCTGC

E A L N Y E T V E Q K K A Y E M S E L L

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Fig.9 continued

1330

1350

1370

TTGGTGACATTGGGGGCCAGATGGGGCTGTTCATCGGGGCCAGCCTGCTCACCATCCTCG

G D I G G Q M G L F I G A S L L T I L E

1390

1410

1430

AGATCCTAGACTACCTCTGTGAGGTGTTCCGAGACAAGGTCTGGATATTCTGGAACC

I L D Y L C E V F R D K V L G Y F W N R

Fig.10 a

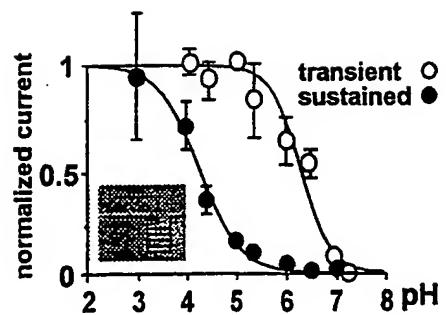
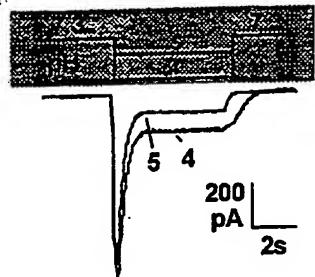


Fig.10 b

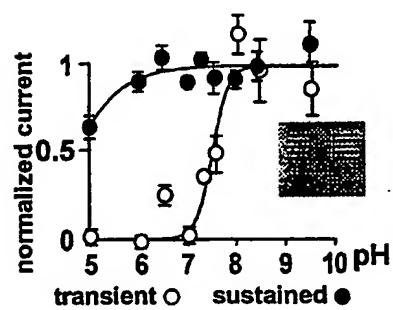
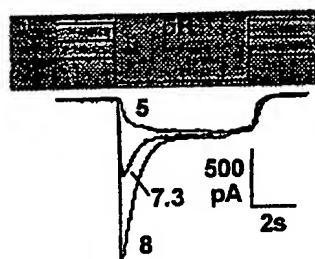


Fig.10 c

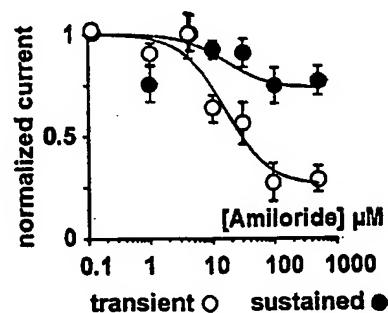
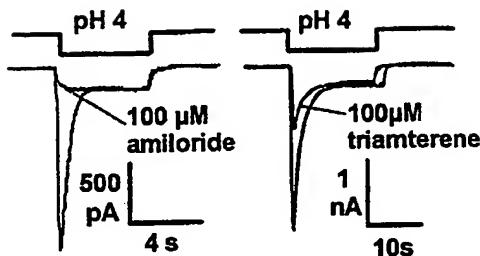


Fig.11 a

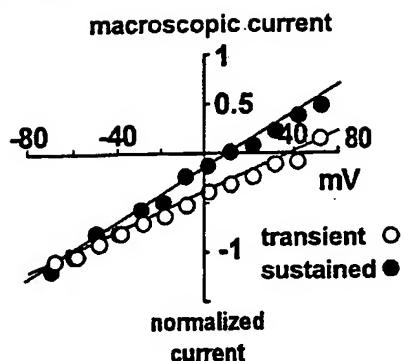


Fig.11 b

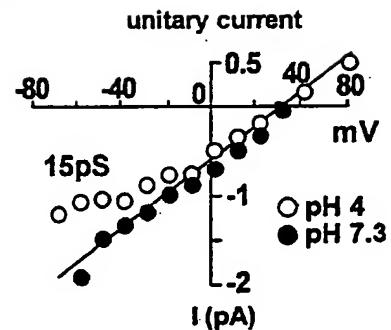


Fig.11 c

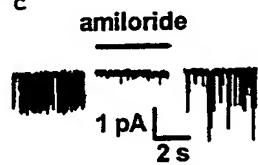
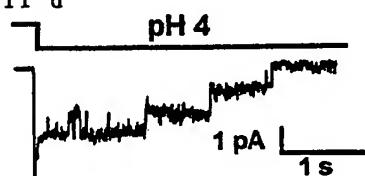


Fig.11 d



17/17

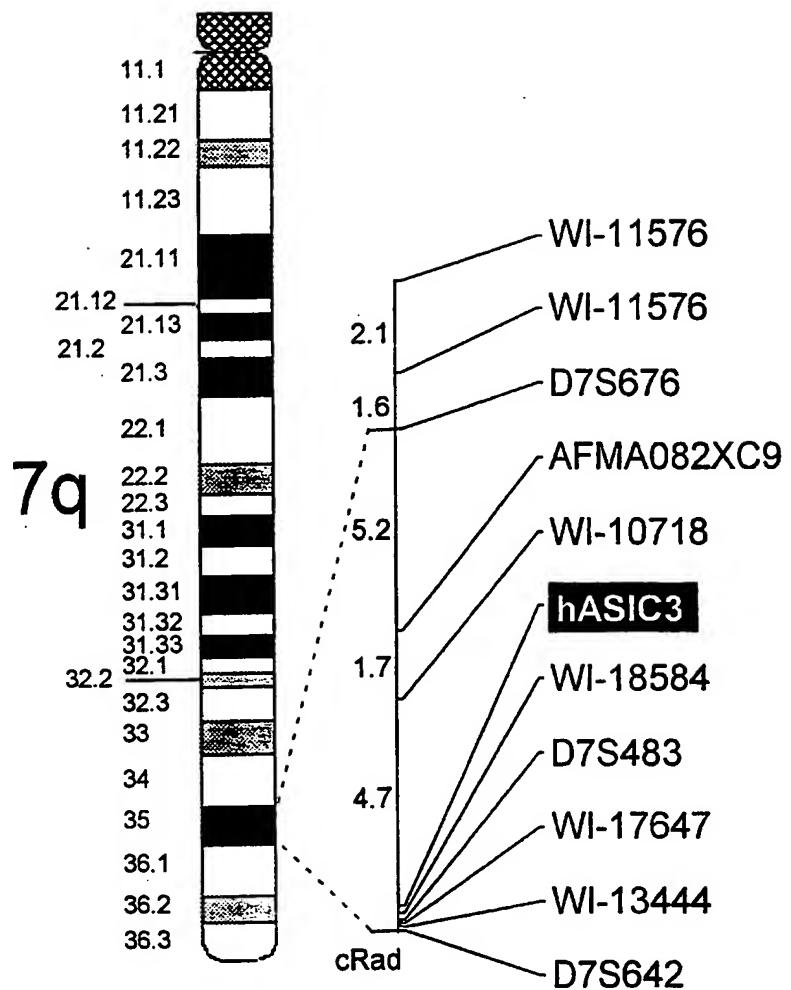


Figure 12

LISTE DE SÉQUENCES.

NOMBRE DE SÉQUENCES : 8

5 INFORMATION CONCERNANT LA SEQ ID NO:1 :

10 i) CARACTRISTIQUE DE LA SEQUENCE :
A) LONGUEUR : 3562 paires de base
B) TYPE : acide nucléique
C) NOMBRE DE BRINS : double
D) CONFIGURATION : linéaire

15 ii) TYPE DE MOLECULE : ADN
 vi) ORIGINE : rat

 ix) CARACTERISTIQUE
 A) NOM/CLE : ASIC
 B) LOCALISATION : 123 .. 1700

20 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:1 :

25	CACACACACA CACACACACA CACACACACA CACACACACA CACACAGAAC CTGCGCCTGT GCCGTGCCT GTGCCTGTGC CTGTTGAGA GCTGGAGACA CAGAAGGATC CCCTTGGCAA GG ATG GAA TTG AAG ACC GAG GAG GAG GTG GGT GGT GTC CAG CCG Met Glu Leu Lys Thr Glu Glu Glu Val Gly Val Gln Pro 1 5 10 15	60 120 167
30	GTG AGC ATC CAG GCT TTC GCC AGC AGC TCC ACG CTG CAT GGT CTT GCC Val Ser Ile Gln Ala Phe Ala Ser Ser Thr Leu His Gly Leu Ala 20 25 30	215
35	CAC ATC TTC TCC TAT GAG CGG CTG TCT CTG AAG CGG GCA CTG TGG GCC His Ile Phe Ser Tyr Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala 35 40 45	263
40	CTG TGC TTC CTG GGT TCG CTG GCC GTC CTG CTG TGT GTG TGC ACT GAG Leu Cys Phe Leu Gly Ser Leu Ala Val Leu Leu Cys Val Cys Thr Glu 50 55 60	311
45	CGT GTG CAG TAC TAC TTC TGC TAT CAC CAC GTC ACC AAG CTT GAC GAA Arg Val Gln Tyr Tyr Phe Cys Tyr His His Val Thr Lys Leu Asp Glu 65 70 75	359
50	GTG GCT GCC TCC CAG CTC ACC TTC CCT GCT GTC ACA CTG TGC AAT CTC Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn Leu 80 85 90 95	407
55	AAT GAG TTC CGC TTT AGC CAA GTC TCC AAG AAT GAC CTG TAC CAT GCT Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu Tyr His Ala 100 105 110	455
55	GGG GAG CTG CTG GCC CTG CTC AAC AAC AGG TAT GAG ATC CCG GAC ACA Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp Thr 115 120 125	503

CAG ATG GCT GAT GAA AAG CAG CTA GAG ATA TTG CAG GAC AAG GCC AAC						551	
Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn							
130 135 140							
5	TTC CCG AGC TTC AAG CCC AAG CCC TTC AAC ATG CGT GAA TTC TAC GAC						599
	Phe Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp						
	145 150 155						
10	AGA GCG GGG CAC GAT ATT CGA GAC ATG CTG CTC TCG TGC CAC TTC CGT						647
	Arg Ala Gly His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg						
	160 165 170 175						
15	GGG GAG GCC TGC AGC GCT GAA GAT TTC AAA GTG GTC TTC ACT CGG TAT						695
	Gly Glu Ala Cys Ser Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr						
	180 185 190						
	GGG AAG TGT TAC ACA TTC AAC TCG GGC CAA GAT GGG CGG CCA CGG CTG						743
	Gly Lys Cys Tyr Thr Phe Asn Ser Gly Gln Asp Gly Arg Pro Arg Leu						
	195 200 205						
20	AAG ACC ATG AAA GGT GGG ACT GGC AAT GGC CTG GAG ATC ATG CTG GAC						791
	Lys Thr Met Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp						
	210 215 220						
25	ATT CAG CAA GAT GAA TAT TTG CCT GTG TCG GGA GAG ACC GAC GAG ACA						839
	Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu Thr						
	225 230 235						
30	TCC TTC GAA GCA GGC ATC AAA GTG CAG ATC CAC AGT CAG GAT GAA CCC						887
	Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu Pro						
	240 245 250 255						
35	CCT TTC ATC GAC CAG CTG GGC TTT GGT GTG GCT CCA GGT TTC CAG ACG						935
	Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr						
	260 265 270						
	TTT GTG TCT TGC CAG GAG CAG AGG CTC ATC TAC CTG CCC TCA CCC TGG						983
	Phe Val Ser Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp						
	275 280 285						
40	GGC ACC TGC AAT GCT GTC ACC ATG GAC TCG GAT TTC TTC GAC TCC TAC						1031
	Gly Thr Cys Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr						
	290 295 300						
45	AGC ATC ACT GCC TGC CGG ATT GAT TGC GAG ACG CGT TAC CTG GTG GAG						1079
	Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu						
	305 310 315						
50	AAC TGC AAC TGC CGT ATG GTG CAC ATG CCA GGG GAC GCC CCA TAC TGC						1127
	Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys						
	320 325 330 335						
55	ACT CCA GAG CAG TAC AAG GAG TGT GCA GAT CCT GCC CTG GAC TTC CTA						1175
	Thr Pro Glu Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu						
	340 345 350						
	GTG GAG AAA GAC CAG GAA TAC TGC GTG TGT GAG ATG CCT TGC AAC CTG						1223
	Val Glu Lys Asp Gln Glu Tyr Cys Val Cys Glu Met Pro Cys Asn Leu						
	355 360 365						

370	375	380	1271
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10	GGG GAG AAC ATT CTG GTG CTG GAC ATT TTC TTT GAA GTC CTC AAC TAT Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val Leu Asn Tyr 400 405 410 415		1367
15	GAG ACC ATC GAG CAG AAA AAG GCC TAT GAG ATC GCA GGG CTG TTG GGT Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu Gly 420 425 430		1415
20	GAC ATC GGG GGC CAG ATG GGG TTG TTC ATC GGT GCC AGC ATC CTC ACC Asp Ile Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr 435 440 445		1463
25	GTG CTG GAA CTC TTT GAC TAT GCC TAC GAG GTC ATT AAG CAC AGG CTG Val Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu 450 455 460		1511
30	TGC AGA CGT GGA AAG TGC CAG AAG GAG GCT AAG AGG AGC AGC GCA GAC Cys Arg Arg Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp 465 470 475		1559
35	AAG GGC GTG GCG CTC AGC CTG GAT GAC GTC AAA AGA CAC AAT CCC TGC Lys Gly Val Ala Leu Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys 480 485 490 495		1607
40	GAG AGC CTC CGA GGA CAT CCT GCC GGG ATG ACG TAC GCT GCC AAC ATC Glu Ser Leu Arg Gly His Pro Ala Gly Met Thr Tyr Ala Ala Asn Ile 500 505 510		1655
45	CTA CCT CAC CAT CCC GCT CGA GGC ACG TTT GAG GAC TTT ACC TGC TAA Leu Pro His His Pro Ala Arg Gly Thr Phe Glu Asp Phe Thr Cys 515 520 526		1703
50	GCCCTCGCAG GCCGCTGTAC CAAAGGCCTA GGTGGGGAGG GCTGGGGAGG CAAGGGGCC CCAACTGCC CCAAGCTACCC TGTGGACTTA ACTGCATTCC TGTCAGTGG TTCCCTCTTIG TCTGTGGTGA GAAAGGAGTC TTGACCATAG AGTCCTCTCC CAGCCTCTAT CCCATCTTTT TATTTTAATT TAATCACATT TGCTCTGTAA TATTGCTGAA GGCTGGGGAT CGTGATTTCC CCCCAGTTCT TTTATTGTTG AGAATAGTTT TCTCTATTCT GGGTTTTCTG TTATTTCAAA TGAATCTGCA AATTGCTCTT CCCATCTCTA TGAAGAATTG CGTTGGAATT TTGATGGGA TTGTATTGAA TCTGTAGATT GCCTTGGTA AGATGCCAT TTTTACTATG TTAATCCTGC CAATTCTATGA GCAAGGGAGA TCTTTCTATC TCTGAAATCT ACTTCAGTTT CTTTCTTCAG AGACTTGAAG TTCTTGTCA AAAAACTTTT TTGGTTAGAG CCACACCAAG GTATTTATA TTGTTTGTGA CTATTGTGAA TGGTGTCAATT TCCCTAATT CTTTCTCAGC CTACTTATCC	1763 1823 1943 2003 2063 2123 2183 2243 2303 2363	1823 1943 2003 2063 2123 2183 2243 2303 2363

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	AAGTTCTTTA TCAGGTTTAG GTGTTCTCTG GTGGAACTTT TGGGGTCACG TAAGAATACT	2483
5	ATTATATCAT CTGCAAATAG TGATATTCA CTTCTCCCTT TCCAATTCT ATCCCTCTGG	2543
	GGACTTTTG TTGTCTAATT GCTCTGGCTA GGACTTCAAA TTCTATATTG AATAGATAGG	2603
10	GAGAGAGTGG GCAGCCTTGT CTAGTCCCTG GTTTTCTGG GATCGCTTC AATTCTCTC	2663
	CATTAGTTT GATATTGGCT ACTGGTTGC TGTATATGGC TTTTACTGTA CTTAGGTATG	2723
	GGCCTGAAAT TCCIGATAATT TCCAAGACTT TTAACATGAA GGGGTTTGA AATTGCAA	2783
15	ATGCTTTCTC AGCATCTAAT GAGATGATCA TGTGCCCTCC CCCCACCTTG AGTTTGTAA	2843
	TATAGTGGGT TACATGAAAG GATCATTCT AATAGTCCAC AAGTCTGCCA AATCTTGCTG	2903
20	ATTGTGACTC ATTTCCATAG CAGGCTCTAT AACTCTCTA ACAGATGCA TTAAACTCTG	2963
	CTTGGGAAAG GCATTACCTC TTGGTTGAAG CAATGTTGTA GTTTCTATGC CTGCTGAGTA	3023
	AATAGCCTCA AGTCCAAGTA CTTGCCAGA CTAATGATCA AACGTATCCA GGAGTTCCAT	3083
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	AGAGATACTC CTPCGAACTG CAAAAGAAAT CTCTGGCTA AGCATATAAT CAAGCCTCAG	3203
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	GGAGACAGAA TGACTCTGTG CCTTCACTGA TGGAAGTCTG GGTTACAAAT TACATCAGAA	3323
	GAACCTATCA TAGTGAAACA TCTCATTCCC CTGGTATAAT CCCTCTAGA AATACACTTG	3383
35	TGACTCTGAA ATGTTATAAT CGTGACAACT AGGCTGTTAC AGATACACCA AGTTAAATTT	3443
	GATAGAGAAA CCAGGCTTGG AGCCTCATGT CCATAGGGCA AGAGGAAGAT GCTGAGTGT	3503
40	TAAGGTTGGT TTGAGCGAAG AACAAATACCT TGTGTCACAA AAATGAAAGG AAAAAAGAAA	3563
	AAAGGAAAGA AGGAAAGAAA GAGAGAGAAA GAAAAAGAAA GAAAGAAAAA AAAAAAAA	3562

INFORMATION CONCERNANT LA SEO ID NO:2 :

5	i) CARACTRISTIQUE DE LA SEQUENCE :	
	A) LONGUEUR : 1620 paires de base	
	B) TYPE : acide nucléique	
	C) NOMBRE DE BRINS : double	
	D) CONFIGURATION : linéaire	
10	ii) TYPE DE MOLECULE : ADN	
	vi) ORIGINE : homme	
	ix) CARACTERISTIQUE	
	A) NOM/CLE : ASIC	
	B) LOCALISATION : 1 .. 1542	
15	xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:2 :	
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	GCC CAC ATC TTC TCC TAC GAG CGG CTG TCT CTG AAG CGG GCA CTG TGG Ala His Ile Phe Ser Tyr Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp	96
	20 25 30	
25	GCC CTG TGC TTC CTG GGC TCG CTG GCT GTG CTG CTG TGT GTG TGC ACG Ala Leu Cys Phe Leu Gly Ser Leu Ala Val Leu Leu Cys Val Cys Thr	144
	35 40 45	
30	GAG CGT GTG CAG TAC TAC TTC CAC TAc CAC CAT GTC ACC AAG CTC GAC Glu Arg Val Gln Tyr Tyr Phe His Tyr His His Val Thr Lys Leu Asp	192
	50 55 60	
35	GAG GTG GCT GCC TCT CAG CTT ACC TTC CCT GCT GTC ACG CTG TGC AAC Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn	240
	65 70 75 80	
40	CTC AAC GAG TTC CGC TTT AGC CAA GTC TCC AAG AAT GAC CTG TAT CAT Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu Tyr His	288
	85 90 95	
45	GCT GGG GAG CTG CTG GCC CTG CTC AAC AAC AGG TAT GAG ATA CCA GAC Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp	336
	100 105 110	
	ACA CAG ATG GCA GAT GAA AAG CAG CTG GAG ATA CTG CAG GAC AAA GCC	384
	Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala	
	115 120 125	
50	AAC TTC CGC AGC TTC AAA CCC AAA CCC TTC AAC ATG CGT GAG TTC TAC Asn Phe Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr	432
	130 135 140	
55	GAC CGA GCT GGG CAC GAC ATT CGA GAC ATG CTG CTC TCC TGC CAC TTC Asp Arg Ala Gly His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe	480
	145 150 155 160	

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25	CCT CCT TTC ATC GAC CAG CTG GGC TTT GGC GTG GCC CCA GGC TTC CAG Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln 245 250 255	768
30	ACC TTT GTG GCC TGC CAG GAG CAG CGG CTC ATA TAC CTG CCC CCA CCC Thr Phe Val Ala Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Pro Pro 260 265 270	816
35	TGG GGC ACC TGC AAA GCT GTT ACC ATG GAC TCG GAT TTG GAT TTC TTC Trp Gly Thr Cys Lys Ala Val Thr Met Asp Ser Asp Leu Asp Phe Phe 275 280 285	864
40	GAC TCC TAC AGC ATC ACT GCC TGC CGC ATC GAC TGT GAG ACG CGC TAC Asp Ser Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr 290 295 300	912
45	CTG GTG GAG AAC TGC AAC TGC CGC ATG GTG CAC ATG CCA GGG GAT GCC Leu Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala 305 310 315 320	960
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60	TGC AAC CTG ACC CGC TAT GGC AAA GAG CTG TCC ATG GTC AAG ATC CCC Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met Val Lys Ile Pro 355 360 365	1104
65	AGC AAA GCC TCA GCC AAG TAC CTG GCC AAG AAG TTC AAC AAA TCT GAG Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu 370 375 380	1152
70	CAA TAC ATA GGG GAG AAC ATC CTG GTG CTG GAC ATT TTC TTT GAA GTC Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val 385 390 395 400	1200

	CTC AAC TAT GAG ACC ATT GAA CAG AAG GGC TAT GAG ATT GCA GGG Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly 405 410 415	1248
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10	ATC CTC ACG GTG CTG GAG CTC TTT GAC TAC GCC TAC GGG GTC ATT AAG Ile Leu Thr Val Leu Glu Leu Phe Asp Tyr Ala Tyr Gly Val Ile Lys 435 440 445	1344
15	CAC AAG CTG TGC CGA CGA GGA AAA TGC CAG AAG GAG GCC AAA AGG AGC His Lys Leu Cys Arg Arg Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser 450 455 460	1392
20	AGT GCG GAC AAG GGC GTG GCC CTC AGC CTG GAC GAC GTC AAA AGA CAC Ser Ala Asp Lys Gly Val Ala Leu Ser Leu Asp Asp Val Lys Arg His 465 470 475 480	1440
25	AAC CCG TGC GAG AGC CTT CGG GGC CAC CCT GCC GGG ATG ACA TAC GCT Asn Pro Cys Glu Ser Leu Arg Gly His Pro Ala Gly Met Thr Tyr Ala 485 490 495	1488
30	GCC AAC ATC GTA CCT CAC CAT CCG GCC CGA GGC ACG TTC GAG GAC TTT Ala Asn Ile Val Pro His His Pro Ala Arg Gly Thr Phe Glu Asp Phe 500 505 510	1536
	ACC TGC TGA GCCCCGCAGG CCGCCGAACC AAAGACCTAG ATGGGGAGGA CTAGGAGAGC Thr Cys *	1595
	GAGGGGGCCC CCAGCTGCCT CCTAA	1620

35

INFORMATION CONCERNANT LA SEQ ID NO:3 :

5 i) CARACTRISTIQUE DE LA SEQUENCE :
 A) LONGUEUR : 1666 paires de base
 B) TYPE : acide nucléique
 C) NOMBRE DE BRINS : double
 D) CONFIGURATION : linéaire

10 ii) TYPE DE MOLECULE : ADN
 10 vi) ORIGINE : homme

15 ix) CARACTERISTIQUE
 A) NOM/CLE : MDEG
 B) LOCALISATION : 127 .. 1663

15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:3 :

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	GGTCCCCG	ATG GAC CTC AAG GAA AGC CCC AGT GAG GGC AGC CTG CAA CCT					169									
	Met	Asp	Leu	Lys	Glu	Ser	Pro	Ser	Glu	Gly	Ser	Leu	Gln	Pro		
25	1	5	10													
	TCT AGC ATC CAG ATC TTT	GCC AAC ACC TCC ACC CTC CAT GGC ATC CGC					217									
	Ser	Ser	Ile	Gln	Ile	Phe	Ala	Asn	Thr	Ser	Thr	Leu	His	Gly	Ile	Arg
30	15	20	25	30												
	CAC ATC TTC GTG TAT GGG CCG CTG ACC ATC CGG CGT GTG CTG TGG GCA							265								
	His	Ile	Phe	Val	Tyr	Gly	Pro	Leu	Thr	Ile	Arg	Arg	Val	Leu	Trp	Ala
35	35	40	45													
	GTG GCC TTC GTG GGC TCT CTG GGC CTG CTG GTG GAG AGC TCT GAG							313								
	Val	Ala	Phe	Val	Gly	Ser	Leu	Gly	Leu	Leu	Leu	Val	Glu	Ser	Ser	Glu
40	50	55	60													
	AGG GTG TCC TAC TAC TTC TCC TAC CAG CAT GTC ACT AAG GTG GAC GAA							361								
	Arg	Val	Ser	Tyr	Tyr	Phe	Ser	Tyr	Gln	His	Val	Thr	Lys	Val	Asp	Glu
45	65	70	75													
	GTG GTG GCT CAA AGC CTG GTC TTC CCA GCT GTG ACC CTC TGT AAC CTC							409								
	Val	Val	Ala	Gln	Ser	Leu	Val	Phe	Pro	Ala	Val	Thr	Leu	Cys	Asn	Leu
50	80	85	90													
	AAT GGC TTC CGG TTC TCC AGG CTC ACC ACC AAC GAC CTG TAC CAT GCT							457								
	Asn	Gly	Phe	Arg	Phe	Ser	Arg	Leu	Thr	Thr	Asn	Asp	Leu	Tyr	His	Ala
55	95	100	105	110												
	GGG GAG CTG CTG GCC CTG CTG GAT GTC AAC CTG CAG ATC CCG GAC CCC							505								
	Gly	Glu	Leu	Leu	Ala	Leu	Leu	Asp	Val	Asn	Leu	Gln	Ile	Pro	Asp	Pro
55	115	120	125													
	CAT CTG GCT GAC CCC TCC GTG CTG GAG GCC CTG CGG CAG AAG GCC AAC							553								
	His	Leu	Ala	Asp	Pro	Ser	Val	Leu	Glu	Ala	Leu	Arg	Gln	Lys	Ala	Asn
	130	135	140													

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5	CGT GTG GGC CAT GAC CTG AAG GAT ATG ATG CTC TAC TGC AAG TTC AAA Arg Val Gly His Asp Leu Lys Asp Met Met Leu Tyr Cys Lys Phe Lys 160 165 170	649
10	GGG CAG GAG TGC GGC CAC CAA GAC TTC ACC ACA GTG TTT ACA AAA TAT Gly Gln Glu Cys Gly His Gln Asp Phe Thr Thr Val Phe Thr Lys Tyr 175 180 185 190	697
15	GGG AAG TGT TAC ATG TTT AAC TCA GGC GAG GAT GGC AAA CCT CTG CTC Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp Gly Lys Pro Leu Leu 195 200 205	745
20	ACC ACG GTC AAG GGG GGG ACA GGC AAC GGG CTG GAG ATC ATG CTG GAC Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp 210 215 220	793
25	ATT CAG CAG GAT GAG TAC CTG CCC ATC TGG GGA GAG ACA GAG GAA ACG Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly Glu Thr Glu Glu Thr 225 230 235	841
30	ACA TTT GAA GCA GGA GTG AAA GTT CAG ATC CAC AGT CAG TCT GAG CCA Thr Phe Glu Ala Gly Val Lys Val Gln Ile His Ser Gln Ser Glu Pro 240 245 250	889
35	CCT TTC ATC CAA GAG CTG GGC TTT GGG GTG GCT CCA GGG TTC CAG ACC Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr 255 260 265 270	937
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45	GGT GAG TGC CGA TCC TCA GAG ATG GGC CTC GAC TTT TTT CCT GTT TAC Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp Phe Phe Pro Val Tyr 290 295 300	1033
50	AGC ATC ACC GCC TGT AGG ATT GAC TGT GAG ACC CGC TAC ATT GTG GAA Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Ile Val Glu 305 310 315	1081
55	AAC TGC AAC TGC CGC ATG GTT CAC ATG CCA GGG GAT GCC CCT TTT TGT Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Phe Cys 320 325 330	1129
60	ACC CCT GAG CAG CAC AAG GAG TGT GCA GAG CCT GCC CTA GGT CTG TTG Thr Pro Glu Gln His Lys Glu Cys Ala Glu Pro Ala Leu Gly Leu Leu 335 340 345 350	1177
65	GCG GAA AAG GAC AGC AAT TAC TGT CTC TGC AGG ACA CCC TGC AAC CTA Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg Thr Pro Cys Asn Leu 355 360 365	1225
70	ACC CGC TAC AAC AAA GAG CTC TCC ATG GTG AAG ATC CCC AGC AAG ACA Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys Thr 370 375 380	1273

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	385 390 395	
5	TCA GAG AAC ATC CTT GTT CTG GAT ATA TTT TTT GAA GCT CTC AAT TAT	1369
	Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe. Glu Ala Leu Asn Tyr	
	400 405 410	
10	GAG ACA ATT GAA CAG AAG AAG GCG TAT GAA GTT GCT GCC TTA CTT GGT	1417
	Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val Ala Ala Leu Leu Gly	
	415 420 425 430	
15	GAT ATT GGT GGT CAG ATG GGA TTG TTC ATT GGT GCT AGT ATC CTT ACA	1465
	Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr	
	435 440 445	
	ATA CTA GAG CTC TTT GAT TAT ATT TAT GAG CTG ATC AAA GAG AAG CTA	1513
	Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu Ile Lys Glu Lys Leu	
	450 455 460	
20	TTA GAC CTG CTT GCC AAA GAG GAG GAT GAA GGG AGC CAC GAT GAG AAT	1561
	Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu Gly Ser His Asp Glu Asn	
	465 470 475	
25	GTG AGT ACT TGT GAC ACA ATG CCA AAC CAC TCT GAA ACC ATC AGT CAC	1609
	Val Ser Thr Cys Asp Thr Met Pro Asn His Ser Glu Thr Ile Ser His	
	480 485 490	
30	ACT GTG AAC GTG CCC CTG CAG ACG ACC CTG GGG ACC CTG GAA GAA ATA	1657
	Thr Val Asn Val Pro Leu Gln Thr Thr Leu Gly Thr Leu Glu Glu Ile	
	495 500 505 510	
35	GCC TGC TGA Ala Cys *	1666
	512	

INFORMATION CONCERNANT LA SEQ ID NO:4 :

5 i) CARACTRERISTIQUE DE LA SEQUENCE :
 A) LONGUEUR : 3647 paires de base
 B) TYPE : acide nucléique
 C) NOMBRE DE BRINS : double
 D) CONFIGURATION : linéaire

10 ii) TYPE DE MOLECULE : ADN
 vi) ORIGINE : rat

15 ix) CARACTERISTIQUE
 A) NOM/CLE : ASIC1B
 B) LOCALISATION : 109 .. 1785

15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:4 :

CTGCCACAGA	GGCTCTGGTG	AGGAAGGACA	GACAGCTGGA	CCGGCGCAGA	CCTAGCCGAA	60	
20	GTCCAACCTC	CGTCCCTTCT	GGTGGCTTCT	TCCTGTCTCC	TGAACAAG	ATG CCC ATC	117
					Met	Pro Ile	
					1	3	
25	CAG ATC TTT TGT TCT GTG TCA	TTC TCC TCT GGA	GAG GAG GCG CCG GGA				165
	Gln Ile Phe Cys Ser Val	Ser Phe Ser Ser Gly	Glu Glu Ala Pro Gly				
	5	10	15				
30	TCC ATG GCA GAT ATC TGG GGT CCC CAC CAC CAC CGG CAG CAG CAG GAC	Ser Met Ala Asp Ile Trp Gly Pro His His His Arg Gln Gln Asp					213
	20	25	30				
35	AGC TCA GAA TCG GAA GAA GAG GAA GAG AAG GAA ATG GAG GCA GGG TCG	Ser Ser Glu Ser Glu Glu Glu Glu Lys Glu Met Glu Ala Gly Ser					261
	40	45	50				
40	GAG TTG GAT GAG GGT GAT GAC TCA CCT AGG GAC TTG GTG GCC TTC GCC	Glu Leu Asp Glu Gly Asp Asp Ser Pro Arg Asp Leu Val Ala Phe Ala					309
	55	60	65				
45	AAC AGC TGT ACC TTC CAT GGT GCC AGC CAT GTG TTT GTG GAA GGG GGC	Asn Ser Cys Thr Phe His Gly Ala Ser His Val Phe Val Glu Gly Gly					357
	70	75	80				
50	CCA GGG CCA AGG CAG GCC TTA TGG GCA GTG GCC TTT GTC ATA GCA CTG	Pro Gly Pro Arg Gln Ala Leu Trp Ala Val Ala Phe Val Ile Ala Leu					405
	85	90	95				
55	GGT GCC TTC CTG TGC CAG GTC GGG GAC CGC GTT GCT TAT TAC CTC AGC	Gly Ala Phe Leu Cys Gln Val Gly Asp Arg Val Ala Tyr Tyr Leu Ser					453
	100	105	110				
55	TAC CCA CAC GTG ACT TTG CTA GAC GAA GTG GCC ACC ACG GAG CTG GTC	Tyr Pro His Val Thr Leu Leu Asp Glu Val Ala Thr Thr Glu Leu Val					501
	120	125	130				

	TTC CCA GCT GTC ACC TTC TGC AAC ACC AAT GCC GTG CGG TTG TCC CAG Phe Pro Ala Val Thr Phe Cys Asn Thr Asn Ala Val Arg Leu Ser Gln 135 140 145	549
5	CTC AGC TAC CCT GAC TTG CTC TAC CTG GCC CCC ATG CTA GGA CTG GAT Leu Ser Tyr Pro Asp Leu Leu Tyr Leu Ala Pro Met Leu Gly Leu Asp 150 155 160	597
10	GAG AGT GAT GAC CCC GGG GTG CCC CTT GCT CCT CCT GGC CCA GAG GCT Glu Ser Asp Asp Pro Gly Val Pro Leu Ala Pro Pro Gly Pro Glu Ala 165 170 175	645
15	TTC TCC GGG GAG CCT TTT AAC CTC CAT CGT TTC TAT AAT CGC TCT TGC Phe Ser Gly Glu Pro Phe Asn Leu His Arg Phe Tyr Asn Arg Ser Cys 180 185 190 195	693
20	CAC CGG CTG GAG GAC ATG CTG CTC TAT TGT TCC TAC TGT GGG GGC CCC His Arg Leu Glu Asp Met Leu Leu Tyr Cys Ser Tyr Cys Gly Gly Pro 200 205 210	741
25	TGT GGT CCC CAC AAC TTC TCA GTG GTC TTC ACT CGG TAT GGG AAG TGT Cys Gly Pro His Asn Phe Ser Val Val Phe Thr Arg Tyr Gly Lys Cys 215 220 225	789
30	TAC ACA TTC AAC TCG GGC CAA GAT GGG CGG CCA CGG CTG AAG ACC ATG Tyr Thr Phe Asn Ser Gly Gln Asp Gly Arg Pro Arg Leu Lys Thr Met 230 235 240	837
35	AAA GGT GGG ACT GGC AAT GGC CTG GAG ATC ATG CTG GAC ATT CAG CAA Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp Ile Gln Gln 245 250 255	885
40	GAT GAA TAT TTG CCT GTG TGG GGA GAG ACC GAC GAG ACA TCC TTC GAA Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu Thr Ser Phe Glu 260 265 270 275	933
45	GCA GGC ATC AAA GTG CAG ATC CAC AGT CAG GAT GAA CCC CCT TTC ATC Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu Pro Pro Phe Ile 280 285 290	981
50	GAC CAG CTG GGC TTT GGT GTG GCT CCA GGT TTC CAG ACG TTT GTG TCT Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr Phe Val Ser 295 300 305	1029
55	TGC CAG GAG CAG AGG CTC ATC TAC CTG CCC TCA CCC TGG GGC ACC TGC Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp Gly Thr Cys 310 315 320	1077
55	AAT GCT GTT ACC ATG GAC TCG GAT TTC TTC GAC TCC TAC AGC ATC ACT Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr Ser Ile Thr 325 330 335	1125
55	GCC TGC CGG ATT GAT TGC GAG ACG CGT TAC CTG GTG GAG AAC TGC AAC Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn 340 345 350 355	1173

	TGC CGT ATG GTG CAC ATG CCA GGG GAC GCC CCA TAC TGC ACT CCA GAG .	1221
	Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu	
	360 365 370	
5	CAG TAC AAG GAG TGT GCA GAT CCT GCC CTG GAC TTC CTA GTG GAG AAA	1269
	Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys	
	375 380 385	
10	GAC CAG GAA TAC TGC GTG TGT GAG ATG CCT TGC AAC CTG ACC CGC TAC	1317
	Asp Gln Glu Tyr Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr	
	390 395 400	
15	GGC AAG GAG CTG TCC ATG GTC AAG ATC CCA AGC AAA GCC TCC GCC AAG	1365
	Gly Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys Ala Ser Ala Lys	
	405 410 415	
20	TAC CTG GCC AAG AAG TTC AAC AAA TCG GAG CAG TAC ATA GGG GAG AAC	1413
	Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu Gln Tyr Ile Gly Glu Asn	
	420 425 430 435	
25	ATT CTG GTG CTG GAC ATT TTC TTT GAA GTC CTC AAC TAT GAG ACC ATC	1461
	Ile Leu Val Leu Asp Ile Phe Phe Glu Val Leu Asn Tyr Glu Thr Ile	
	440 445 450	
30	GAG CAG AAA AAG GCC TAT GAG ATC GCA GGG CTG TTG GGT GAC ATC GGG	1509
	Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu Gly Asp Ile Gly	
	455 460 465	
35	GGC CAG ATG GGG TTG TTC ATC GGT GCC AGC ATC CTC ACC GTG CTG GAA	1557
	Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val Leu Glu	
	470 475 480	
40	CTC TTT GAC TAT GCC TAC GAG GTC ATT AAG CAC AGG CTG TGC AGA CGT	1605
	Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu Cys Arg Arg	
	485 490 495	
45	GGA AAG TGC CAG AAG GAG GCT AAG AGG AGC AGC GCA GAC AAG GGC GTG	1653
	Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val	
	500 505 510 515	
50	GCG CTC AGC CTG GAT GAC GTC AAA AGA CAC AAT CCC TGC GAG AGC CTC	1701
	Ala Leu Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu	
	520 525 530	
55	CGA GGA CAT CCT GCC GGG ATG ACG TAC GCT GCC AAC ATC CTA CCT CAC	1749
	Arg Gly His Pro Ala Gly Met Thr Tyr Ala Ala Asn Ile Leu Pro His	
	535 540 545	
60	CAT CCC GCT CGA GGC ACG TTT GAG GAC TTT ACC TGC TAA GCCCTCGCAG	1798
	His Pro Ala Arg Gly Thr Phe Glu Asp Phe Thr Cys	
	550 555 559	
65	GCCGCTGTAC CAAAGGCCTA GGTGGGGAGG GCTGGGGAG CAAGGGGCC CCAACTGCC	1858
70	CCAGCTACCC TGTGGACTTA ACTGCATTCC TGGTCAGTGG TTCCCTCTTG TCTGTGGTGA	1918
75	GAAAGGAGTC TTGACCATAG AGTCCTCTCC CAGCCTCTAT CCCATCTTT TATTTTAATT	1978
80	TAATCACATT TGCTCTGAA TATTGCTTGA GGCTGGGGAT CGTGATTTCC CCCCAGTTCT	2038

	TTTATTGTTG AGAATAGTTT TCTCTATTCT GGGTTTTCTG TTATTTCAAA TGAATCTGCA	2098
	AATTGCTCTT CCCATCTCTA TGAAGAATTG CGTTGGAATT TTGATGGGA TTGTATTGAA	2158
5	TCTGTAGATT GCCTTGGTA AGATGCCAT TTTTACTATG TTAATCCTGC CAATTCAATGA	2218
	GCAAGGGAGA TCTTTCTATC TCTGAAATCT ACTTCAGTTT CTTTCTTCAG AGACTTGAAG	2278
	TTCTTGTCAAT AAAAATCTTT TTGGTTAGAG CCACACCAAG GTATTTATA TTGTTTGTGA	2338
10	CTATTGTGAA TGGTGTCAATT TCCCTAATTT CCTTCTCAGC CTACTTATCC TTTGAGTAGA	2398
	GGAAGGCTTC TGATTTGTTT GGGTTAATTT TATAACCCAGC TGCTTTGCTA AAGTCTTTA	2458
15	TCAGGTTTAG GTGTTCTCTG GTGGAACTTT TGGGGTCACG TAAGAATACT ATTATATCAT	2518
	CTGCAAATAG TGATATTCTA CTTCTTCCTT TCCAATTCT ATCCCTCTGG GGACTTTTG	2578
20	TTGTCTAATT GCTCTGGCTA GGACTTCAAA TTCTATATTG AATAGATAGG GAGAGAGTGG	2638
	GCAGCCTTGT CTAGTTCCTG GTTTCTGTTG GATCGCTTCA AATTCTCTC CATTAGTTT	2698
	GATATTGGCT ACTGGTTTGC TGTATATGGC TTTTACTGTA CTTAGGTATG GGCCTTGAAT	2758
25	TCCTGATATT TCCAAGACTT TTAACATGAA GGGGTTTGA AATTGCCAA ATGCTTTCTC	2818
	AGCATCTAAT GAGATGATCA TGTGCCCTCC CCCCACCTTG AGTTTGTAA TATAGTGGGT	2878
	TACATGAAAG GATCATTCT AATAGTCCAC AAGTCTGCCA AATCTTGCTG ATTGTGACTC	2938
30	ATTTCCATAG CAGGCTCTAT AACTCTCTA ACAGATTGCA TTAAACTCTG CTTGGGAAG	2998
	GCATTACCTC TTGGTTGAAG CAATGTTGTA GTTCTATGC CTGCTGAGTA AATAGCCTCA	3058
35	AGTCCAAGTA CTTGCCAGA CTAATGATCA AACGTATCCA GGAGTTCCAT ACCAGAGATG	3118
	TACTCTCTC TCCCTTGAAG TACATTGCTG GAAGAGTAAT TGTGTTTGCT AGAGATACTC	3178
	CTTCGAACTG CAAAAGAAAT CTCTTGGCTA AGCATATAAT CAAGCCTCAG GTTTCTTTT	3238
40	TATTAATAG CTGCTTGTAA GAAAGTGGAC ACTAAGCATA TACCTCAAAG GGAGACAGAA	3298
	TGACTCTGTG CCTTCACTGA TGGAAGTCTG GGTTACAAAT TACATCAGAA GAACCTATCA	3358
45	TAGTGAACCA TCTCATTCCC CTGGTATAAT CCCTTCTAGA AATACACTTG TGACTCTGAA	3418
	ATGTTATAAT CGTGACAACG AGGCTGTTAC AGATACACCA AGTTAAATTT GATAGAGAAA	3478
50	CCAGGCTTGG AGCCTCATGT CCATAGGGCA AGAGGAAGAT GCTGAGTGT TAAGGTTGGT	3538
	TTGAGCGAAG AACAAATACCT TGTGTCACAA AAATGAAAGG AAAAAAGAAA AAAGGAAAGA	3598
	AGGAAAGAAA GAGAGAGAAA GAAAAAGAAA GAAAGAAAAA AAAAAAAA	3647

INFORMATION CONCERNANT LA SEO ID NO:5 :

5	i) CARACTRISTIQUE DE LA SEQUENCE :	
	A) LONGUEUR 1602 paires de base	
	B) TYPE : acide nucléique	
	C) NOMBRE DE BRINS : double	
	D) CONFIGURATION : linéaire	
10	ii) TYPE DE MOLECULE : ADN	
	vi) ORIGINE : rat	
15	ix) CARACTERISTIQUE	
	A) NOM/CLE : DRASIC	
	B) LOCALISATION : 1 .. 1602	
20	xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:5 :	
	ATG AAA CCT CGC TCC GGA CTG GAG GAG GCC CAG CGG CGA CAG GCC TCA Met Lys Pro Arg Ser Gly Leu Glu Glu Ala Gln Arg Arg Gln Ala Ser 1 5 10 15	
25	GAC ATC CGG GTG TTT GCC AGC AGC TGC ACA ATG CAT GGT CTG GGC CAC Asp Ile Arg Val Phe Ala Ser Ser Cys Thr Met His Gly Leu Gly His 20 25 30	
30	ATC TTT GGC CCT GGA GGC CTG ACC CTG CGC CGA GGG CTG TGG GCC ACA Ile Phe Gly Pro Gly Gly Leu Thr Leu Arg Arg Gly Leu Trp Ala Thr 35 40 45	
35	GCT GTG CTC CTG TCG CTG GCG GCC TTC CTC TAC CAG GTG GCT GAG CGG Ala Val Leu Leu Ser Leu Ala Ala Phe Leu Tyr Gln Val Ala Glu Arg 50 55 60	
40	GTT CGC TAC TAT GGG GAG TTC CAC CAT AAG ACC ACC CTG GAT GAG CGT Val Arg Tyr Tyr Gly Glu Phe His His Lys Thr Thr Leu Asp Glu Arg 65 70 75 80	
45	GAG AGC CAC CAG CTC ACC TTC CCA GCT GTG ACT CTG TGT AAT ATC AAC Glu Ser His Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn Ile Asn 85 90 95	
50	CCA CTG CGC CGC TCA CGC CTC ACA CCC AAT GAC TTG CAC TGG GCT GGA Pro Leu Arg Arg Ser Arg Leu Thr Pro Asn Asp Leu His Trp Ala Gly 100 105 110	
55	ACA GCG CTG CTG GGC CTG GAC CCT GCT GAA CAT GCT GCT GCC TAC CTT CGT Thr Ala Leu Leu Gly Leu Asp Pro Ala Glu His Ala Ala Tyr Leu Arg 115 120 125	
60	GCA CTG GGC CAG CCC CCC GCA CCA CCT GGC TTC ATG CCC AGT CCG ACC Ala Leu Gly Gln Pro Pro Ala Pro Pro Gly Phe Met Pro Ser Pro Thr 130 135 140	
65	TTT GAC ATG GCA CAA CTC TAC GCC AGA GCC GGC CAC TCC CTT GAG GAC Phe Asp Met Ala Gln Leu Tyr Ala Arg Ala Gly His Ser Leu Glu Asp 145 150 155 160	

	ATG TTG TTG GAT TGC CGA TAC CGT GGC CAG CCC TGT GGG CCT GAG AAC Met Leu Leu Asp Cys Arg Tyr Arg Gly Gln Pro Cys Gly Pro Glu Asn 165 170 175	528
5	TTC ACA GTG ATC TTT ACT CGA ATG GGG CAA TGC TAC ACC TTC AAC TCT Phe Thr Val Ile Phe Thr Arg Met Gly Gln Cys Tyr Thr Phe Asn Ser 180 185 190	576
10	GGT GCC CAC GGT GCA GAG CTG CTC ACC ACT CCA AAG GGT GGT GCT GGC Gly Ala His Gly Ala Glu Leu Leu Thr Thr Pro Lys Gly Gly Ala Gly 195 200 205	624
15	AAC GGA CTG GAG ATT ATG CTA GAT GTA CAG CAA GAG GAG TAT CTG CCC Asn Gly Leu Glu Ile Met Leu Asp Val Gln Gln Glu Glu Tyr Leu Pro 210 215 220	672
20	ATC TGG AAG GAC ATG GAA GAG ACC CCG TTT GAG GTG GGG ATC CGA GTG Ile Trp Lys Asp Met Glu Glu Thr Pro Phe Glu Val Gly Ile Arg Val 225 230 235 240	720
25	CAG ATT CAC AGC CAG GAT GAG CCC CCT GCC ATT GAC CAG CTG GGC TTC Gln Ile His Ser Gln Asp Glu Pro Pro Ala Ile Asp Gln Leu Gly Phe 245 250 255	768
30	GGG GCA GCC CCA GGC CAT CAG ACT TTT GTG TCC TGT CAG CAG CAA Gly Ala Ala Pro Gly His Gln Thr Phe Val Ser Cys Gln Gln Gln 260 265 270	816
35	CTG AGT TTC CTG CCA CCA CCC TGG GGT GAC TGC AAT ACC GCA TCT TTG Leu Ser Phe Leu Pro Pro Trp Gly Asp Cys Asn Thr Ala Ser Leu 275 280 285	864
40	GAT CCC GAC GAC TTT GAT CCA GAG CCC TCT GAT CCC TTG GGT TCC CCC Asp Pro Asp Asp Phe Asp Pro Glu Pro Ser Asp Pro Leu Gly Ser Pro 290 295 300	912
45	AGA CCC AGA CCC AGC CCT CCT TAT AGT TTA ATA GGT TGT CGC CTG GCC Arg Pro Arg Pro Ser Pro Pro Tyr Ser Leu Ile Gly Cys Arg Leu Ala 305 310 315 320	960
50	TGT GAG TCT CGC TAT GTG GCT CGG AAG TGT GGC TGT CGA ATG ATG CAT Cys Glu Ser Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met Met His 325 330 335	1008
55	ATG CCT GGA AAC TCC CCA GTG TGC AGC CCC CAG CAG TAC AAG GAC TGC Met Pro Gly Asn Ser Pro Val Cys Ser Pro Gln Gln Tyr Lys Asp Cys 340 345 350	1056
60	GCC AGC CCA GCT CTG GAC GCT ATG CTG CGA AAG GAC ACG TGT GTC TGC Ala Ser Pro Ala Leu Asp Ala Met Leu Arg Lys Asp Thr Cys Val Cys 355 360 365	1104
65	CCC AAC CCG TGC GCT ACT ACA CGC TAT GCC AAG GAG CTC TCC ATG GTG Pro Asn Pro Cys Ala Thr Thr Arg Tyr Ala Lys Glu Leu Ser Met Val 370 375 380	1152
70	CGG ATT CCC AGC CGC GCG TCA GCT CGC TAC CTG GCC CGG AAA TAC AAC Arg Ile Pro Ser Arg Ala Ser Ala Arg Tyr Leu Ala Arg Lys Tyr Asn 385 390 395 400	1200

	CGC AGC GAG TCC TAC ATT ACG GAG AAT GTA CTG GTT CTG GAT ATC TTC	1248
	Arg Ser Glu Ser Tyr Ile Thr Glu Asn Val Leu Val Leu Asp Ile Phe	
	405 410 415	
5	TTT GAG GCC CTC AAC TAT GAA GCG GTG GAA CAA AAG GCG GCC TAT GAA	1296
	Phe Glu Ala Leu Asn Tyr Glu Ala Val Glu Gln Lys Ala Ala Tyr Glu	
	420 425 430	
10	GTG TCG GAG CTG CTG GGA GAC ATT GGG GGA CAG ATG GGA CTG TTT ATT	1344
	Val Ser Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile	
	435 440 445	
15	GGA GCA ACC CTG CTT ACC ATC CTT GAG ATC CTC GAC TAT CTC TGT GAG	1392
	Gly Ala Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu Cys Glu	
	450 455 460	
20	GTT TTC CAA GAC AGA GTC CTG GGG TAT TTC TGG AAC AGA AGG AGC GCT	1440
	Val Phe Gln Asp Arg Val Leu Gly Tyr Phe Trp Asn Arg Arg Ser Ala	
	465 470 475 480	
25	CAA AAG CGC TCT GGC AAC ACT CTG CTC CAG GAA GAG TTG AAT GGC CAT	1488
	Gln Lys Arg Ser Gly Asn Thr Leu Leu Gln Glu Glu Leu Asn Gly His	
	485 490 495	
30	CGA ACA CAT GTT CCC CAC CTC AGC CTA GGG CCC AGG CCT CCT ACC ACT	1536
	Arg Thr His Val Pro His Leu Ser Leu Gly Pro Arg Pro Pro Thr Thr	
	500 505 510	
	CCC TGT GCT GTC ACC AAG ACA CTC TCT GCC TCC CAC CGT ACC TGT TAC	1584
	Pro Cys Ala Val Thr Lys Thr Leu Ser Ala Ser His Arg Thr Cys Tyr	
	515 520 525	
35	CTC GTC ACA AGG CTC TAG	1602
	Leu Val Thr Arg Leu *	
	530 533	

INFORMATION CONCERNANT LA SEQ ID NO:6 :

	i) CARACTRISTIQUE DE LA SEQUENCE :	
5	A) LONGUEUR 1948 paires de base	
	B) TYPE : acide nucléique	
	C) NOMBRE DE BRINS : double	
	D) CONFIGURATION : linéaire	
10	ii) TYPE DE MOLECULE : ADN	
	vi) ORIGINE : rat	
15	ix) CARACTERISTIQUE	
	A) NOM/CLE : MDEG2	
	B) LOCALISATION : 16 .. 1707	
20	xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:6 :	
	CCTCGGGCTG AATGA ATG AGC CGG AGC GGC GGA GCC CGG CTG CCC GCG ACC	51
	Met Ser Arg Ser Gly Gly Ala Arg Leu Pro Ala Thr	
	1 5 10	
	GCG CTC AGC GGC CCG GGA CGC TTC CGT ATG GCC CGC GAG CAG CCG GCG	99
	Ala Leu Ser Gly Pro Gly Arg Phe Arg Met Ala Arg Glu Gln Pro Ala	
	15 20 25	
25	CCC GTG GCG GTG GCG GCA GCT AGG CAG CCC GGA GGA GAC CGG AGC GGC	147
	Pro Val Ala Val Ala Ala Arg Gln Pro Gly Gly Asp Arg Ser Gly	
	30 35 40	
30	GAT CCG GCG CTG CAG GGG CCA GGG GTC GCC CGC AGG GGG CGG CCG TCC	195
	Asp Pro Ala Leu Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro Ser	
	45 50 55 60	
35	CTG AGT CGC ACT AAA TTG CAC GGG CTG CGG CAC ATG TGC GCG GGG CGC	243
	Leu Ser Arg Thr Lys Leu His Gly Leu Arg His Met Cys Ala Gly Arg	
	65 70 75	
40	ACG GCG GCG GGA GGC TCT TTC CAG CGA CGG GCG CTG TGG GTG CTG GCC	291
	Thr Ala Ala Gly Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu Ala	
	80 85 90	
45	TTC TGC ACG TCC CTC CGC TTG CTG CTG TCC TGG TCC TCG AAC CGC CTG	339
	Phe Cys Thr Ser Leu Gly Leu Leu Ser Trp Ser Ser Asn Arg Leu	
	95 100 105	
50	CTC TAC TGG CTC AGC TTC CCG TCA CAC ACA CGA GTG CAC CGT GAG TGG	387
	Leu Tyr Trp Leu Ser Phe Pro Ser His Thr Arg Val His Arg Glu Trp	
	110 115 120	
55	AGC CGC CAG CTG CCG TTC CCC GCC GTC ACC GTG TGC AAC AAC AAC CCC	435
	Ser Arg Gln Leu Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn Pro	
	125 130 135 140	
	CTG CGC TTC CCG CGC CTC TCC AAG GGG GAC CTC TAC TAC GCG GGC CAC	483
	Leu Arg Phe Pro Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly His	
	145 150 155	

	TGG CTA GGG CTG CTG CTT CCC AAC CGC ACC GCG CGC CCG CTG GTC AGC Trp Leu Gly Leu Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val Ser 160 165 170	531
5	GAG CTG CTG CGG GCC GAC GAG CCG CGC CGC CAG TGG TTC CGC AAA CTG Glu Leu Leu Arg Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys Leu 175 180 185	579
10	GCC GAC TTC CGC CTC TTC CTG CCG CCG CGC CAC TTC GAG GGC ATC AGC Ala Asp Phe Arg Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile Ser 190 195 200	627
15	GCT GCC TTC ATG GAC CGT TTG GGC CAC CAG CTG GAG GAT ATG CTG CTC Ala Ala Phe Met Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu Leu 205 210 215 220	675
20	TCC TGC AAG TAC CGG GGC GAG CTC TGT GGC CCG CAC AAC TTC TCC TCA Ser Cys Lys Tyr Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser Ser 225 230 235	723
25	GTC TTT ACA AAA TAC GGG AAG TGT TAC ATG TTT AAC TCA GGC GAG GAT Val Phe Thr Lys Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp 240 245 250	771
30	GGC AAG CCG CTG CTC ACC ACG GTC AAG GGG GGG ACG GGC AAC GGG CTG Gly Lys Pro Leu Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu 255 260 265	819
35	GAG ATC ATG CTG GAC ATT CAG CAA GAT GAG TAC CTG CCC ATC TGG GGA Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly 270 275 280	867
40	GAG ACA GAG GAA ACA ACG TTT GAA GCA GGA GTG AAG GTT CAG ATC CAC Glu Thr Glu Glu Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile His 285 290 295 300	915
45	AGT CAG TCT GAG CCG CCT TTC ATC CAA GAG CTG GGC TTT GGG GTG GCT Ser Gln Ser Glu Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala 305 310 315	963
50	CCG GGG TTC CAG ACC TTC GTG GCC ACA CAA GAG CAG AGG CTC ACA TAT Pro Gly Phe Gln Thr Phe Val Ala Thr Gln Glu Gln Arg Leu Thr Tyr 320 325 330	1011
55	CTG CCC CCA CCA TGG GGG GAG TGC CGG TCC TCA GAG ATG GGA CTC GAC Leu Pro Pro Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp 335 340 345	1059
55	TTC TTT CCT GTT TAC AGC ATC ACA GCC TGT CGG ATT GAC TGT GAG ACC Phe Phe Pro Val Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr 350 355 360	1107
55	CGC TAC ATC GTG GAG AAC TGT AAC TGC CGC ATG GTC CAC ATG CCA GGG Arg Tyr Ile Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly 365 370 375 380	1155

	GAC GCC CCT TTC TGC ACC CCT GAG CAG CAC AAG GAG TGT GCA GAG CCT Asp Ala Pro Phe Cys Thr Pro Glu Gin His Lys Glu Cys Ala Glu Pro 385 390 395	1203
5	GCC CTC GGT CTA CTG GCA GAA AAG GAC AGC AAT TAC TGT CTC TGC AGG Ala Leu Gly Leu Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg 400 405 410	1251
10	ACA CCC TGC AAC CTG ACA CGC TAC AAC AAA GAG CTC TCC ATG GTG AAG Thr Pro Cys Asn Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys 415 420 425	1299
15	ATC CCC AGC AAG ACG TCA GCC AAG TAC TTA GAG AAG AAA TTT AAC AAA Ile Pro Ser Lys Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn Lys 430 435 440	1347
20	TCG GAA AAA TAT ATC TCA GAG AAC ATT CTT GTT CTG GAC ATA TTT TTT Ser Glu Lys Tyr Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe 445 450 455 460	1395
25	GAG GCG CTC AAT TAC GAA ACA ATT GAA CAG AAG AAG GCG TAT GAA GTT Glu Ala Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val 465 470 475	1443
30	GCT GCC TTA CTT GGT GAC ATC GGT CAG ATG GGA CTG TTC ATT GGT Ala Ala Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly 480 485 490	1491
35	GCT AGT CTC CTC ACA ATA CTA GAG CTC TTT GAT TAT ATT TAT GAG CTG Ala Ser Leu Leu Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu 495 500 505	1539
40	ATC AAA GAG AAG CTA TTA GAC CTG CTT GGC AAA GAA GAA GAG GAA GGG Ile Lys Glu Lys Leu Leu Asp Leu Leu Gly Lys Glu Glu Glu Gly 510 515 520	1587
45	AGC CAC GAT GAG AAC ATG AGC ACC TGT GAC ACA ATG CCA AAC CAC TCT Ser His Asp Glu Asn Met Ser Thr Cys Asp Thr Met Pro Asn His Ser 525 530 535 540	1635
50	GAA ACC ATC AGC CAC ACT GTG AAC GTG CCC CTG CAG ACA GCT TTG GGC Glu Thr Ile Ser His Thr Val Asn Val Pro Leu Gln Thr Ala Leu Gly 545 550 555	1683
	ACC CTG GAG GAG ATT GCC TGC TGA CACCTCTCAG GCAACGCAGC ACCTCCAAAC Thr Leu Glu Ile Ala Cys * 560 563	1737
	AGACCTTAAA GGCCCAAGAC CTAGGACAGG AGACAGCAAG CGCAGGTGGG ATCGCCCCCTG ACGACTGAAA GAAGCAGAGC CCCCCATATG CACACATTGC GAACTTCTGC CAAACCTCAC CTGGCCACAT CTGACATGAA CCGTCCCGGG CCCTGCGTCA TGTCCTCGC AGGACCGATG AGTCGCACTC CGGAACGTGTC CAAGAACTAA C	1797 1857 1917 1948

SEQ ID No. 7

ACGACGGGGTTCTGGCCATGAAGCCCACCTCAGGCCAGAGGAGGCCGGCGGCCAGCCTCGGACATCCCGCGTGGTCGCCAGCAACTGCTCGATGCACGGGCTGGGCCACGTCTTCGGGC CAGGCAGCCTGAGCCTGCCGGGGATGTGGGAGCAGGCCGTGGCTCTGTCAAGTGGCCA CCTTCCCTCTACCAAGGTGGCTGAGAGGGTGCCTACTACAGGGAGTCCACCACAGACTGCCCTGGATGAGCGAGAAAGCCACCGCTCATCTTCCCAGCTGTCAACCATCAACCCACTGCGCCGCTCGGCCCTAACGCCAACGACCTGCACTGGGCTGGGTCTGCGCTGC TGGGCTGGATCCCGCAGAGCACGCCGCCTTCTGCGCCGCCTGGGCCGGCCCCCTGCAC CGCCCGCTTCACTGCCAGTCCCACCTTGACATGGCGCAACTCTATGCCGTGCTGGGACTCCCTGGATGACATGCTGCTGGACTGTCGCTCCGGCAACCTGTGGGCTGAGA ACTTCACCACGATCTTCAACCGGATGGGAAAGTGTACACATTTAACTCTGGCGCTGATGGGGCAGAGCTGCTCACCAACTAGGGGTGGCATGGGCAATGGGCTGGACATCATGCTGGACGTGCAAGCAGGAGGAATATCTACCTGTGTGGAGGGACAATGAGGAGACCCCGTTGAGG TGGGATCCGAGTGCAGATCCACAGCCAGGAGGCCGGCATCATCGATCAGCTGGGCTTGGGTGTCCCCGGCTACCAAGACCTTGTGTCAGCAGCAGCAGCTGAGCTTCTGCCACCGCCCTGGGGCGATTGCAAGTTCAGCATCTCTGAACCCCAACTATGAGCCAGAGC CCTCTGATCCCCTAGGCTCCCCAGCCCCAGCCCCAGCCTCCCTATACCTTATGGGGTGTGCCCTGGCTCGAAACCCGCTACGTGGCTCGGAAGTGCAGCTGCCGAATGGTGTACA TGCCAGGCAGTGCAGTGTGCAAGGACTCGTGCCTGCCAACCCGTGCCAGCACGCGCTAGATGCCATGCCATGCTCGCAAGGACTCGTGCCTGCCAACCCGTGCCAGCACGCGCTAGGCCAACCCGCTCGAACGGAGCTCTCCATGGTGCCTGGGATCCCCAGCCGCGCCGCTTCCCTGGCCC GGAAGCTCAACCGCAGCGAGGCCTACATCGGGAGAACGTGCTGGCCATGGACATCTCTTGAGGCCCTCAACTATGAGACCCTGGAGCAGAAGAACGGCTATGAGATGTCAGAGCTGCTTGTTGACATTGGGGCCAGATGGGCTGTTCATGGGCCAGCCTGCTCACCATCCTCGAGATCCTAGACTACCTCTGTGAGGTGTTCCGAGACAAGGTCTGGATATTCCTGGAAACCGACAGCACTCCAAAGGCACTCCAGCACCAATCTGCTTCAGGAAGGGCTGGCAGCCATCGAAGCCAAAGTCTCCACCTCAGCCTGGGCCAGACCTCCACCCCTCCCTGTGCCGTCA CCAAGACTCTCTCCGCCTCCCACCCGACCTGCTACCTTGTCACACAGCTCTAGACCTGCTGTCTGCTCGCTAGCCTGCACTGGACATGCCCTGCTCACGCTAGCTTTTCCGTCTTCACCCCAAATAAGTCTTAATGCATCAAAAAAAAAAAAAAA

SEQ ID No. 8

M	K	P	T	S	G	P	E	A	R	P	I	R	V	F	A	S
N	C	S	M	H	G	L	H	V	A	G	S	L	R	R	Y	M
W	A	A	A	V	V	L	V	A	L	F	F	A	E	V	P	Y
Y	R	E	F	H	H	Q	T	A	R	E	E	Y	L	R	A	Y
V	T	L	C	N	I	P	A	R	R	S	S	Q	D	I	P	Y
G	S	A	L	L	G	D	P	A	A	H	T	Q	A	L	Q	A
P	A	P	P	G	F	M	P	T	F	F	D	P	G	V	G	P
H	S	T	L	D	D	M	L	C	R	F	R	G	D	Q	F	G
T	T	T	I	F	T	R	M	G	K	F	F	L	G	V	Q	H
L	Y	L	P	V	W	G	D	M	G	N	I	E	G	S	S	F
S	Q	E	E	P	P	P	I	I	E	Q	T	P	P	S	P	P
V	S	C	Q	Q	Q	Q	Q	P	S	F	L	G	P	S	S	P
L	N	P	N	Y	E	M	G	E	P	S	D	P	G	A	C	C
P	Y	M	T	L	M	P	C	R	L	A	C	V	P	R	C	H
R	M	V	Y	Y	M	G	G	D	V	S	C	S	P	K	N	R
P	A	I	D	A	A	L	M	R	K	D	S	A	C	A	S	R
Y	A	K	E	L	S	M	G	V	R	I	P	S	A	A	S	G
L	N	R	S	E	A	Y	I	R	A	E	N	V	L	E	L	C
N	Y	E	T	V	E	Q	K	A	K	A	Y	E	M	L	I	I
Q	M	G	L	F	I	G	A	S	S	R	V	E	I	Q	Q	I
V	F	R	D	K	V	L	G	Y	F	W	N	R	H	H	S	H
N	L	L	Q	E	G	G	S	H	R	T	Q	S	S	R	S	G
P	P	T	P	P	C	A	V	T	K	T	L	S	R	T	T	V
T	Q	L	Q	L												